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EVALUATION OF GERMICIDAL HAND WASH AGENTS FOR USE IN FOOD HANDLING

by

ARIF Z. SHEENA

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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DEDICATION

TO THE MEMORY OF MY PARENTS

ABSTRACT

The efficacy of hygienic hand wash procedures for food handlers, using germicidal hand washes and dips, was studied by measuring changes in numbers of microorganisms released from hands before and after 15-s wash treatments. The 4% chlorhexidine gluconate and iodophor (0.75% available iodine) products were the only agents that gave significant decreases in numbers of bacteria released from hands. Products containing 0.25% Irgasan DP 300 (2-4-4'trichloro-2'-hydroxy diphenyl ether), 0.5% tribromosalicylanilide or 0.325% *para*-chloro-*meta*-xylenol (PCMX) were no better than the non-germicidal soap against the resident microflora. The Quaternary Ammonium (QAC) hand dip was the only hand dip that gave a reduced count. Identification of 3,591 aerobic isolates from the bacterial plates indicated that *Staphylococcus epidermidis* and *Micrococcus* spp. were the predominating organisms (85.3%) released from the hands. The agents were also tested for immediate and residual (substantive) germicidal effect against the residual hand microflora. Chlorhexidine gluconate (4%) liquid detergent gave an immediate and substantive reduction in number of microorganisms released. The iodophor product (0.75% available iodine) gave comparable results for the immediate reduction of microorganisms released, but it did not give a substantive effect. Other products failed to give an immediate or a substantive reduction in microorganisms released.

The efficacy of germicidal hand wash agents was also tested against transient bacteria (*Escherichia coli* and *Pseudomonas fluorescens*) inoculated into ground beef and rubbed onto the hands. Most of the agents tested, including 4% chlorhexidine gluconate, iodophor (0.75% available iodine), Irgasan DP 300, PCMX as well as the non-germicidal soap gave marked reduction in the counts of *E. coli* and *P. fluorescens* (>90% reduction) after one 15-s wash. The hand dip treatments with iodophor, hypochlorite or QAC were generally less effective than hand wash treatments, especially against *P. fluorescens*. The 4% chlorhexidine gluconate and iodophor (0.75% available iodine) products gave significantly greater decrease in number of *E. coli* on hands, compared to the other agents. Two barrier creams for use on hands were compared with germicidal agents to determine their ability to reduce the number of microorganisms released from skin. Barrier creams resulted in a significant decrease in the number of microorganisms released, equivalent to the reduction achieved when 4% chlorhexidine gluconate or iodophor (0.75% available iodine) were used. Sequential rinsing of hands with tap water, after treatment with the barrier creams or with the effective germicidal agents, gave similar results.

A final study was done to determine the germicidal efficacy of low and intermediate strength iodophor products and a 2% chlorhexidine gluconate product. The efficacy was measured against transient bacteria (*Escherichia coli* and

Pseudomonas fluorescens) inoculated onto hands, and the resident-type microflora on hands. Only 4% chlorhexidine gluconate and iodophor (0.75% available iodine) gave a significantly better reduction of the transient bacteria than other agents. However, all other agents, including non-germicidal soap were significantly better than the tap water rinse. The data for the resident-type bacteria were less distinct, but they indicated that many of the agents were better than the tap water rinse, non-germicidal soap and the lower concentration iodophor products.

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I. INTRODUCTION

A. Microbiology of the Skin

Human skin is a good medium and environment for the growth of certain types of microorganisms. Price (1938a) divided skin bacteria into two groups: transient and resident flora. He defined the transient flora as those microorganisms acquired from extraneous sources, superficially located on the skin, easily removed by washing, relatively scarce on clean, unexposed skin, while abundant on exposed skin. They can be pathogenic or non-pathogenic. The resident flora were defined as the relatively stable microbial population, more deeply situated on the skin, less susceptible to removal by washing, and that can grow on the skin. More recently, Somerville-Millar and Noble (1974), added a category of skin bacteria that they refer to as "nomads", to include those bacteria that might be only temporarily resident on the skin, or that might represent repeated contamination from external sources.

It was shown by Kligman (1965) that the aerobic staphylococci and diphtheroid bacteria formed the majority of the resident flora of normal skin, while gram-negative bacilli, such as *Mima*, *Herellea* and *Aerobacter* formed the minor components of this flora. Woodroffe and Shaw (1974) indicated that errors could be made in assigning an organism to the resident or transient flora. Such errors have been

ascribed to: differences in sampling methods for skin bacteria, complexities of the natural features of the skin, and the fact that some species of skin microorganisms require special culture techniques.

There have been several reviews of the literature on the normal flora of human skin (Evans *et al.*, 1950; Kligman, 1965; Marples, 1969, 1974; Noble and Somerville, 1974). The dominant resident flora of the skin are cited as genera of the family *Micrococcaceae*, in particular staphylococci and *Micrococcus* spp., lipophilic and non-lipophilic diphtheroids and gram-negative bacilli, which may achieve resident status on some humid areas of the skin, such as the axillae (Woodroffe and Shaw, 1974).

The susceptibility of the skin surface to different microorganisms is influenced by several external factors, including: age, sex, race, climate, occupation, health and hygiene (Kligman, 1965). Blank (1965) cited several internal factors that affect the survival of microorganisms on skin, including: degree of hydration and water availability, pH, presence of toxic metabolites and concentration of inorganic salts. He reported that any of these factors can cause the death of skin bacteria. The degree of hydration also affects the proportions of the different bacteria on the skin (Marples, 1965; Woodroffe and Shaw, 1974). An increased level of hydration resulted in an increase in diphtheroids at the expense of the *Micrococcaceae* and gram-negative bacilli.

Nutrients on the skin surface are derived from extraneous sources, as well as from apocrine and eccrine sweat, the *stratum corneum*, and the lipid secretions of the sebaceous glands (Noble and Somerville, 1974; Woodroffe and Shaw, 1974). Microbial population density and antagonism between the different skin microorganisms are important factors determining their ecology. Inhibition may be attributed to bacteriocins produced by the resident flora, but it may also be attributed to phage, antibiotics, bacteriolytic enzymes and depletion of essential nutrients (Wright and Terry, 1981). Some fatty acids act as inhibitors, discouraging the colonization of undesirable microorganisms on the skin.

Different regions of the skin support different types and densities of microorganisms. However, the same types of microorganisms occur over most areas of the skin. Location of bacteria on skin was studied by Kligman (1965), and localization of the resident flora was shown to be in the depth of the hair follicles. Studies using light and electron microscopy (Montes and Wilborn, 1969) revealed that bacteria were located on the skin surface, beneath the superficial cells of the *stratum corneum*, and that numerous gram-positive cocci were located at the opening of the hair follicles. Studies on human and pig skin (Baxby and Woodroffe, 1965) supported the fact that not only the follicles, but also the glands, acted as reservoirs, providing a continuous supply of bacteria to the skin

surface. Their study indicated that bacteria were concentrated in the surface layers, and that their numbers became progressively less in the deep layers of the skin.

Marples (1974) reviewed the literature on the location of the cutaneous flora, and concluded that the principal foci for skin microorganisms are the superficial layers of the *stratum corneum* and the upper portion of the hair follicles. Somerville (1969a,b) studied the normal flora of the skin of different age groups, and observed that coagulase negative staphylococci and micrococci occurred frequently on all sites of all subjects; however, streptococci were also found on infant skin, diphtheroid bacteria on the skin of adults, and both diphtheroid bacteria and strptococci were found on the skin of older people. Noble (1969) studied the distribution of *Micrococcaceae* on the skin, based on Baird-Parker's (1963) taxonomic classification. The schema enabled a breakdown of the coagulase-negative, gram-positive, catalase-positive cocci, formerly considered as a single group into several subgroups (species) of *Staphylococcus* and *Micrococcus*.

The literature on the incidence of gram-negative bacteria on skin has been reviewed (Knittle *et al.*, 1975; Lowbury, 1969; Noble and Savin, 1971; Noble and Somerville, 1974). From these reviews it was concluded that gram-negative bacteria are rare on normal skin, although various species can be isolated sporadically. Gram-negative bacteria have been isolated from the skin of children

(Somerville, 1969a). In a study of 30 normal subjects, Lowbury (1969) reported that 50 percent carried gram-negative bacilli among the skin flora. *Pseudomonas aeruginosa* is considered a transient microorganism on skin. Infections due to this, and other gram-negative organisms, are common in hospitals. Nurses and other hospital personnel have been shown to carry such organisms on their hands (Bruun and Solberg, 1973; Knittle *et al.*, 1975; Lowbury, 1969). *P. aeruginosa* is currently one of the most important bacteria in nosocomial infections (Lowbury, 1969; Noble, 1981).

The gram-negative enteric bacteria, such as *Escherichia coli*, *Proteus* spp., *Klebsiella* spp. and *Enterobacter* spp. can be present on the skin as part of the transient flora, usually as a result of self-contamination from the intestine (Williams, 1965). These bacteria are poorly adapted to the skin (Kligman, 1965), and hospital acquired infections are usually caused by antibiotic resistant *E. coli* and *Klebsiella* spp. (Salzman *et al.*, 1968). It was reported by these workers that 20 percent of hospital personnel carry antibiotic resistant coliform bacteria on their hands. Casewell and Phillips (1977) reported that klebsiellae survived for up to 150 min. on artificially contaminated hands, and they concluded that hands are an important route of transmission in hospital acquired *Klebsiella* infection of obscure epidemiology.

Other bacteria associated with skin include the diphtheroids, streptococci, *Bacillus* spp., aerobic and anaerobic corynebacteria, *Staphylococcus aureus* and yeasts and moulds. *Corynebacterium acnes* has been reported to be dispersed all over the skin surface (Kligman, 1965). The most common site of the lipophilic diphtheroids is the anterior nares (Somerville, 1973). Streptococci, especially the β-haemolytic group, do not form part of the normal skin flora. However, other groups of streptococci may be found in certain sites or in certain groups of individuals, especially infants (Noble and Somerville, 1974; Somerville, 1969a). Aerobic spore forming bacteria have been reported in small numbers on the skin of certain individuals (Kligman, 1965; Marples, 1974), especially as one of the variable types of bacteria isolated from children (Somerville, 1969a).

Williams (1965) reviewed the information available on pathogenic microorganisms found on the skin. *S. aureus* was the only accepted pathogenic bacterium that occurred naturally on the skin. The carriage of *S. aureus* on the skin was reviewed by Williams (1963). Noble *et al*. (1967) studied the carriage of *S. aureus* by a random sample of a normal european population. They indicated that the nose, throat and fingers were sites for carriage of *S. aureus*. Williams (1969) stated that a large proportion of *S. aureus* found on the skin were transient microorganisms that originated from the nose. In a study of 26 subjects from whom *S. aureus* had

been recovered (Voss, 1975), it was found that approximately 60 percent of the subjects were nasal carriers of *S. aureus*, confirming the relationship between nasal carriage and occurrence of *S. aureus*.

Yeasts and moulds are also found on the skin. Moulds such as *Aspergillus* spp. and *Penicillium* spp. are widespread in the environment and are considered to be transient microorganisms on the skin (Noble and Somerville, 1974). *Candida albicans*, is commonly associated with the microflora of the mouth. It is not able to colonize dry skin (Noble, 1981).

There is a scarcity of information on the carriage of viruses on the skin. Viruses have been recorded on skin in cases of viral disease (Noble and Somerville, 1974).

B. Hand Wash Agents

Personal hygiene is an important aspect of public health. It is primarily based on frequent cleaning of the skin. The necessity of hand washing to maintain desirable levels of hygiene is generally accepted. The use of a wide range of soaps, detergent preparations and germicidal agents has been recommended over a long period of time. Soaps prepared from different fatty acids differ markedly in germicidal properties, while sodium or potassium salts of the same fatty acids do not differ in their germicidal activity (Walker, 1924). One of the most effective commercial soaps against gram-negative bacteria is soap

prepared exclusively from coconut oil. The thorough washing of hands with the formation of a good lather removes a large number of different types of bacteria from skin (Walker, 1925). The importance of soap in removal of recent skin contaminants was cited by Bettley (1960).

Different types of liquid soaps were studied by Ojajärvi (1981). Little difference was observed in the total number of bacteria, *S. aureus*, and gram-negative bacilli on the hands as a result of using different soaps. Soap acceptability by personnel was cited as an important factor in promoting proper hand washing and hygiene. Non-germicidal or non-medicated soaps are usually included in experimental trials as negative controls for comparison with germicidal agents (Ayliffe *et al.*, 1975; Bibel, 1977; Joress, 1962; Jungermann and Taber, 1971; Lilly and Lowbury, 1971; Wilson, 1970).

Alcohol is a widely accepted germicidal agent. It has been used to disinfect wounds, and as a degerming agent on hands and on skin of operating sites (Hatfield and Lockwood, 1943; Lowbury *et al.*, 1974). The use of 70% ethanol was claimed by Price (1939) to be more effective than 95% ethanol, while Hatfield and Lockwood (1943) reported that 95% ethanol for 1 min. exposure was more effective. Nonetheless, ethanol is an effective skin germicide, and its use in conjunction with other germicidal agents has been shown to be highly effective, for example: 0.5% chlorhexidine in 70% or 95% ethanol (Ayliffe *et al.*, 1975;

Lowbury *et al.*, 1974); also 0.5% chlorhexidine in 70% isopropanol (Aly and Maibach, 1979, 1980; Lowbury *et al.*, 1974).

Hexachlorophene was probably the most widely used skin germicide. It received wide acceptance despite comparative studies indicating that it was not as effective as some other agents (Davies *et al.*, 1977; Joress, 1962; MacPherson *et al.*, 1965; Peterson *et al.*, 1978; Smylie *et al.*, 1973). However, Gibson (1969) reported that in a soap gel, hexachlorophene was effective against several types of microorganisms, especially gram-positive cocci. Although Weatherall and Winner (1963) reported that intermittent use of a 2% hexachlorophene soap gave no improvement over non-germicidal soap, the repetitive use of hexachlorophene gave improved results compared to non-germicidal soaps. This residual or substantive effect of this agent was widely reported (Hall, 1980; Smylie *et al.*, 1973; Van der Hoeven and Hinton, 1968).

Hexachlorophene was considered to be relatively non-toxic, until its dermal absorption in infants was reported (Curley *et al.*, 1971), and it was shown that extensive absorption and storage of the hexachlorophene in fat tissue may cause fatal encyphalopathy (Chilcote *et al.*, 1977). Many governments changed the license for use of hexachlorophene, and in Canada and other countries the maximum allowable use is 0.75% without a medical prescription (National Health and Welfare, 1980). The

adverse publicity and the reduced allowable use concentration in non-prescription items has lead to a decrease in use of hexachlorophene, and alternate agents are being used as active ingredients in germicidal soaps.

A 4% chlorhexidine digluconate preparation in detergent solution has been described as one of the most effective, immediate skin degerming agents, and it is reported to maintain its efficacy upon repeated use (Hall, 1980). The use of chlorhexidine and its microbicidal effect on different types of bacteria and fungi was reviewed by Beeuwkes (1958). He reported that this agent had a broad antibacterial spectrum, as well as fungistatic and fungicidal activity for various medical purposes. Chlorhexidine has been used and compared with other germicidal agents (Davies *et al.*, 1977; Ojajärvi, 1976; Peterson *et al.*, 1978; Smylie *et al.*, 1973), it has also been studied in different formulations and concentrations (Aly and Maibach, 1979, 1980; Lilly and Lowbury, 1971; Lowbury *et al.*, 1974). Chlorhexidine has a broad spectrum antibacterial action, which includes *S. aureus* and *P. aeruginosa*. The safety of chlorhexidine has also been evaluated (Butler and Iswaran, 1980) and no indication of toxicity or hazard for man was suggested. A 4% chlorhexidine solution is mostly used in hospitals and laboratories for hygienic disinfection (Ojajärvi, 1976), however its use in alcoholic formulation as a surgical scrub is also recommended (Ayliffe *et al.*, 1975; Lowbury *et al.*, 1974).

Irgasan DP 300 (2-4-4'trichloro-2'-hydroxy diphenyl ether), also called triclosan, is another germicidal agent, which has been introduced in the last decade (Bodey and Rosenbaum, 1973). It has been reported to be active against gram-positive and gram-negative microorganisms (Anonymous, 1979; Furia and Schenkel, 1968). Irgasan DP 300 has been used in comparative studies with other germicidal agents at 2% concentration or less, and it has generally been shown to be less effective than 0.5% alcoholic chlorhexidine and 4% chlorhexidine liquid detergent (Ayliffe *et al.*, 1975) and to have a relatively small residual effect upon repetitive use (Lowbury *et al.*, 1974; Ojajärvi, 1976). However, 2% Irgasan DP 300 showed a significant reduction in total numbers of bacteria on hands after six applications, compared to a non-germicidal control soap (Ayliffe *et al.*, 1975). A bar soap incorporating 1% Irgasan DP 300 and 1% tribromosalicylanilide has been reported to be very effective (Bodey and Rosenbaum, 1973).

A phenolic compound, chloroxylenol, has also been studied for use in germicidal agents. The use of *para*-chloro-*meta*-xylenol (PCMX, the active ingredient of dettol) was reported by Colebrook and Maxted (1933). It was shown to be active against haemolytic streptococci and *E. coli*. It has also been reported to be active against *S. aureus* (Beath, 1943; Colebrook, 1941) and against *Pseudomonas* spp. (Lowbury, 1951). However, in comparisons of 2.5% PCMX in aqueous solution with other germicidal agents,

PCMX was less active than most of the agents tested, including alcoholic preparations of 0.5% chlorhexidine, 4% chlorhexidine gluconate liquid detergent and povidone iodine (iodophor) containing 0.75% available iodine (Lowbury *et al.*, 1974).

Other antibacterial agents for use as germicides are the salicylanilides and carbanilides. These agents were reviewed by Stecker (1977) and their efficacy has been studied by other workers (Duncan *et al.*, 1969; Jungermann, 1968; Kooistra *et al.*, 1966; Lemaire *et al.*, 1961; Molnar and Baron, 1964; Stecker and Faust, 1960; Vinson *et al.*, 1961). One of the more popular germicides within this family of chemicals is 3,4,4'-trichlorocarbanilide (TCC). TCC has been incorporated at different concentrations, with and without other agents in soap, to determine the most effective antimicrobial product (Jungermann and Taber, 1971; MacKenzie, 1970). TCC was compared with unmedicated soap (Wilson, 1970), and 2% TCC was found to give a significant reduction of the number of microorganisms on the skin. In contrast, Bibel (1977) reported no significant difference in the number of microorganisms on the skin when using 1.5% TCC or unmedicated soap, however an alteration in the skin microflora was reported with TCC, resulting in increased numbers of *Acinetobacter calcoaceticus* and *Micrococcus luteus*.

Iodophors are widely used for disinfection, and they are also used as skin germicides. Iodophors are produced by

using polyvinyl-pyrrolidone (PVP) as the carrying agent for the iodine, and they are referred to as Povidone or PVP iodine. The chemical, physiological, pharmacological and toxicological aspects of these agents, in comparison with traditional iodine compounds, were originally reviewed by Shelanski and Shelanski (1956). Iodophors have been used in different concentrations for hygienic disinfection of hands of hospital personnel, as well as for food handlers in food service establishments. Povidone iodine containing 0.75% available iodine has been used as a surgical hand scrub and for degerming operation sites (Joress, 1962; Smylie *et al.*, 1973). Many trials have been done including Povidone iodine with 0.75% available iodine and other germicidal agents such as chlorhexidine in alcoholic or detergent formulation, hexachlorophene, Irgasan DP 300 and Quaternary Ammonium compounds (Davies *et al.*, 1977; Joress, 1962; Peterson *et al.*, 1978; Van der Hoeven and Hinton, 1968). In most of these studies, the chlorhexidine and iodophor preparations gave significantly better decrease in the skin microflora. No residual effect was reported with the repetitive use of iodophor (Peterson *et al.*, 1978; Smylie *et al.*, 1973).

Hypochlorite has also been used for hygienic hand disinfection. Although it is most commonly used for water treatment and swimming pools, Krusé (1980) reported the use of hypochlorite as a hand dip at 200 p.p.m. available chlorine, after washing with a non-germicidal soap.

Quaternary Ammonium compounds (QAC) are widely used for disinfection and sanitizing of inanimate surfaces in food handling areas. These products are not commonly used as hand germicides, but they have been included in some comparative studies with other hand germicides (Bruun *et al.*, 1968; Joress, 1962). However, QAC's are generally considered to be poor detergent and germicidal agents for use on the skin (Blank and Coolidge, 1950). This has been attributed to the electrical charge on the keratin surface of the skin. Bacteria are held onto the skin when the charge is positive and released when the charge is negative (Blank and Coolidge, 1950).

C. Methods for Evaluation of Hand Wash Agents

There are two basic approaches for determining the efficacy of germicidal hand wash agents, *in vitro* and *in vivo* tests. Although the simplest method of evaluating antimicrobial agents is by *in vitro* testing, for example the phenol coefficient, the results do not bear any relationship to these agents when they are tested *in vivo*. This is attributed to the fact that the skin has a marked effect on germicidal efficacy. Therefore, practical *in vivo* test results are essential to account for such variables as hydration of the skin, skin secretions and natural inhibitors.

In vivo methods generally involve the application of the germicide to the skin, followed by microbiological

sampling. This is achieved either by applying the germicides to the skin for certain exposure times and conditions, followed by different ways of microbiological sampling (Marples and Kligman, 1974; Quinn *et al.*, 1954; Selwyn and Ellis, 1972; Smylie *et al.*, 1959; Wilson, 1970), or by excising pieces of skin as a biopsy (Selwyn and Ellis, 1972). Nungester and Kempf (1942) used a bizarre technique in which pathogenic bacteria were applied to the tails of mice. The tails were treated with disinfectants, the end of the tail was amputated and implanted into the peritoneal cavity of the animal. Effectiveness of the disinfectants was measured by the mortality rate of the mice.

Price (1938a) developed the multiple basin technique, in an effort to quantitate the number of microorganisms on skin. Hands were scrubbed in a uniform manner using non-germicidal soap and seven different basins, one for each of the seven successive washes. He reported a successive decrease in the number of microorganisms released from hands, and when the log of the cumulative total of microorganisms released from hands was plotted against time (represented by the number in the sequence of basins) a linear relationship was reported. Although linearity has subsequently been disproved (Hurst *et al.*, 1960), this study by Price (1938a) formed the basis of much of the research on hand and skin germicides that followed. Cade (1950, 1952) modified the multiple basin technique to test germicidal soaps. Subjects used the test soap for a specified period (1

to 2 weeks), and then used the control (non-germicidal) soap in the laboratory to collect rinse waters for bacteriological sampling. Cade (1950) defined the degerming action of germicidal soaps as the combined effect of killing the bacteria, removing them mechanically from the skin and suppressing their multiplication.

Many studies have been done using the Price-Cade multiple basin technique or various modifications (Hurst *et al.*, 1960; Peterson, 1972; Quinn *et al.*, 1954; Van der Hoeven and Hinton, 1968; Wilson, 1970). Many of these modifications involved the "standardized" washing procedure. However, Quinn *et al.* (1954) developed the split-use procedure, in which two basins were used for each washing, one for each hand. In this way, each subject served as their own control in the experiment. One hand was washed at a time, while the other was covered by a glove. Because of the virtually simultaneous use of non-germicidal control and germicidal test soaps, this method was considered to be highly sensitive for efficacy testing. This was subsequently handled by use of the Latin Square statistical design (Hurst *et al.*, 1960; Lowbury *et al.*, 1960, 1963) in which each subject used each of the test and control soaps over the course of the experiment.

Hurst *et al.* (1960) made further modifications to the Price-Cade technique, in which subjects used a control soap at least three times daily for one week, before commencing a trial. Subjects were divided into two groups, during the

second week one group used a germicidal soap while the other group used the control soap. During the third week the groups were reversed. Data were analyzed on the basis of percentage reduction over the week of using the germicidal soap, and by calculating the percentage reduction for the two groups and pooling the means. This method still only allowed one germicidal soap to be tested at a time. Hurst *et al.* (1960) introduced the use of a Latin Square design which allowed several soaps to be compared at once rather than sequentially. An Open Cross Over design was used by Dineen (1978) in which subjects used a non-germicidal soap on day 1 of the experiment, they used a germicidal agent on days 3 and 4, and 'crossed over' to another germicidal agent on days 5 and 6. This design might be suitable for certain studies, but it lacks the flexibility of the Latin Square design.

In studies by Lilly, Lowbury and co-workers (Lilly and Lowbury, 1971, 1974, 1978; Lowbury *et al.*, 1960; Lowbury and Lilly, 1973) short exposure times for germicidal hand washing were used to study the efficacy of the agents for hand hygiene of nurses and hospital personnel. These studies contrasted markedly with studies of hand and skin disinfection for surgical use and wound disinfection. Short exposure studies are more appropriate to considerations of hand hygiene for food handlers. In general, exposure times varied from 15 sec to 2 min (Ayliffe *et al.*, 1975; Dineen, 1978; Ojajärvi, 1976). Exposure times in excess of 30 sec

would generally be considered excessive in hand hygiene for food handlers.

Methods of microbiological sampling of hands after washing also vary between studies. Direct sampling by finger tip imprint onto an agar surface has been used, for example by Ojajärvi, (1980) and Sprunt *et al.* (1973). A finger tip streak plating technique has also been used (Ayliffe *et al.* 1975; Smylie *et al.*, 1959, 1973) in which finger and thumb tips are drawn across the surface of an agar plate. Ayliffe *et al.* (1975) recommended this method for use with transient microorganisms. Other methods included swabbing of the skin surface, followed by agitation of the swab in a broth for plating onto agar plates (Story, 1952); adhesive tape applied to the skin to remove microorganisms and then applied to an agar surface (Wilson, 1970); and rinsing the hands in a sampling fluid in a plastic bag (Salzman *et al.*, 1968; Sprunt *et al.*, 1973). An adaptation of the plastic bag technique was the use of plastic gloves for hand sampling (Aly and Maibach, 1979; Dineen, 1978; Lowbury and Lilly, 1960; Michaud *et al.*, 1972; Peterson *et al.*, 1978), using different sampling fluids to rinse the hands. Peterson (1972) used the Quinn *et al.* (1954) split-use method, but, after washing, a sterile surgical glove was placed on the hand and 75 ml of sampling fluid added into ther glove. The gloved hand was massaged for one minute, thereafter the fluid from the glove was used for microbiological testing.

Selwyn and Ellis (1972) did a comparative study of several hand sampling techniques including: direct contact plates, velvet pad transfer, self-adhesive tapes, standardized swabbing, cylinder scrub techniques, as well as excising of skin specimens. They reported that the different sampling techniques yielded different percentages of bacteria compared to counts from biopsies: by scrub techniques 4 to 15% of accessible bacteria were recovered, by swab techniques 3 to 20%, adhesive tape <0.5%, direct contact <0.2% and velvet pad <0.1%.

For indirect sampling techniques (plastic bags, plastic gloves or stainless steel bowls) a rinse fluid is required. A range of solutions has been used, including physiological saline solution (Lowbury and Lilly, 1960; Michaud *et al.*, 1972), Ringer's solution (Ayliffe *et al.*, 1975; Lowbury *et al.*, 1960), 0.1% Triton X-100 in phosphate buffer (Aly and Maibach, 1979; Marples and Kligman, 1974), 10% nutrient broth in saline (Ojajärvi, 1980; Wilson, 1970) and trypticase soy broth (Sprunt *et al.*, 1973). Depending on the germicidal agent used, it might be necessary to neutralize the germicidal agent. Neutralizers are added either to the rinse fluid used to sample the hands and/or to the bacteriological plating medium used to grow the organisms. A range of neutralizers at varying concentrations have been used by different researchers, for example Tween 80, lecithin, Lubrol W and sodium thiosulphate (Aly and Maibach, 1979; Ayliffe *et al.*, 1975; Lowbury *et al.*, 1960; Marples

and Kligman, 1974; Smylie *et al.*, 1973; Sprunt *et al.*, 1973).

Marples and Kligman (1974) developed a more definitive *in vivo* testing procedure, which did not simulate practical use conditions. The agents were applied topically to the skin of the volar (lower surface) forearm. The treatment site was covered with 5 cm^2 of plastic film, and sealed with impermeable plastic tape to occlude the area. Skin that is occluded so that moisture cannot evaporate, develops an ideal environment for growth of the resident skin microflora. Within 48 h the number of microorganisms on occluded skin increases from 100/cm^2 to 10^5/cm^2. In the occlusion test, effective skin germicides prevent the growth of the resident microflora, giving a bacteriostatic effect. Marples and Kligman (1974) also developed the Expanded Flora test. For this test the skin was occluded prior to the application of the antibacterial agents, so that the ability of the agent to decrease the number of microorganisms on the skin could be determined.

Other workers added "transient" microorganisms to the skin, and then tested the germicidal efficacy of the agents, for example by applying bacterial suspensions to small areas of the forearm (Gardner, 1948; Gardner and Seddon, 1946; Lowbury *et al.*, 1960, 1964a; Story, 1952; Wedderburn, 1960) or by applying the bacteria to the hands (Dineen, 1978; Koller *et al.*, 1978; Peterson *et al.*, 1978; Rotter *et al.*, 1974), then carrying out the standardized hand washing

procedures. In these studies the type of transient microorganism and the method of inoculation varied, depending on the interests and purpose of the study. However, there has been a trend to inoculate hands using material that forms the natural substrate for the microorganisms. Sprunt *et al.*, (1973) used soiled diapers in a neonatal nursing unit as a means of contaminating hands with transient microorganisms, and Ojajärvi (1980) used gauze moistened with a bacterial suspension for a laboratory study, and in a clinical study used moist contamination from dressings or compresses and dry contamination from patients cloths and bedding as the source of contamination with transient microorganisms.

The types of bacteria used for the transient inoculum has also varied. *Staphylococcus aureus* (Lilly and Lowbury, 1978) and *Serratia marcescens* (Aly and Maibach, 1980; Peterson *et al.*, 1978) as single bacterium inocula. Ayliffe *et al.* (1978) used a range of single cultures to contaminate hands, including: *E. coli*, *P. aeruginosa*, *S. marcescens*, *Staphylococcus saprophyticus*, *S. aureus* and a *Klebsiella* isolated from a urological ward. They observed that *S. aureus* and the *Klebsiella aerogenes* survived better on the skin than the other bacteria, making them a more significant challenge for hand disinfection studies. *E. coli* also remained constant after an initial decrease during a 2 min. drying period. Other researchers have used multiple strains of bacteria as inocula. Dineen (1978) used a mixture of gram

negative bacilli, including: *E. coli*, *Providencia stuartii*, *P. aeruginosa* and *S. marcescens*, but concentrated on *P. stuartii* and *S. marcescens* because of their particular concern in their institution.

The method of deposition of transient contaminating microorganisms on hands was studied by Koller *et al.* (1978) and Lilly and Lowbury (1978). The comparisons of the inoculation of the bacterial suspension onto each finger tip, followed by rubbing the inocula against the thumb and air drying (as proposed in the guidelines of the Deutsche Gesellschaft für Hygiene und Mikrobiologie), with a hand immersion technique (Koller *et al.*, 1978) revealed that despite differences in the number of bacteria released from finger tips, the results of the efficacy tests were not significantly different between methods of deposition on the hands.

There are no standard *in vivo* techniques for evaluating the efficacy of germicidal hand wash agents. As a result, the methods established for this study were based on the research studies reported in this review of the literature. Because the orientation of the study was to reduce the microbial contamination of foods, the criterion of microbiological efficacy was the ability of the germicidal agent to reduce the number of microorganisms released from hands after washing. Hence, the Price-Cade (multiple basin sampling) technique was not suited to this study. Indirect

sampling by hand rinsing in a plastic bag, and direct sampling by finger tip imprint onto an agar surface, were used in this study, where appropriate.

The experiments were based on the Latin Square design (Myers, 1972) allowing simultaneous comparison of several agents. Neutralization was achieved by the use of letheen broth (Difco) as the sampling fluid for hand rinsing, and letheen agar (Difco) was used for the finger tip imprint technique. These media contain Tween 80 and lecithin as neutralizers.

The study included experiments on both resident and transient types of microorganisms. In the case of transient microorganisms, *Escherichia coli* and *Pseudomonas fluorescens* were used because of their significance to public health and spoilage aspects of foods, respectively. These organisms were suspended in ground beef as the vehicle for contamination of the hands. The specific methods used for each study are outlined in the appropriate chapter. The chapters represent separate research publications (published or submitted for publication) to the Journal of Food Protection or the Journal of Hygiene, Cambridge, with minor modifications to fit the thesis format.

II. OBJECTIVES OF THE STUDY

The principal objective of this study was to evaluate commercially available, germicidal hand wash agents for their ability to reduce the number of microoganisms released from hands after short-exposure, hygienic hand washing. The study was divided into five parts, each part having a specific objective.

1. A range of germicidal hand wash agents and disinfecting hand dip techniques was tested for their ability to reduce the number of resident microorganisms released from hands.

2. Since only the iodophor hand wash agent containing 0.75% available iodine and the 4% chlorhexidine gluconate liquid detergent effectively reduced the number of resident microorganisms released from hands, this study was designed to compare the immediate and residual (substantive) efficacy of Irgasan DP300 and *para*-chloro-*meta*-xylenol (PCMX) products compared to chlorhexidine gluconate (4%) liquid detergent.

3. The germicidal hand wash agents and disinfecting hand dip techniques were tested for their ability to reduce transient (*E. coli* and *P. fluorescens*) microorganisms suspended in ground beef and inoculated onto the hands.

4. The possible use of commercial barrier creams to reduce the number of microorganisms released from hands was

studied by comparison with a non-germicidal liquid hand soap, iodophor containing 0.75% available iodine and chlorhexidine gluconate (4%) liquid detergent.

5. Since the iodophor hand wash agent containing 0.75% available iodine and the chlorhexidine gluconate (4%) liquid detergent remained the only hand wash agents effectively reducing resident and transient microorganisms released from hands, a final study was undertaken to determine whether specially prepared, intermediate and lower concentration iodophor products and a 2% chlorhexidine gluconate product would be as capable of reducing the number of microorganisms released from hands.

III. EFFICACY OF GERMICIDAL HAND WASH AGENTS IN HYGIENIC HAND DISINFECTION

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A. Introduction

Many food industries use germicidal hand washing, especially for workers handling ready-to-eat foods. These agents are used either as a hand dip, in which hands are placed in a prepared germicidal solution, or as hand washes using liquid or solid germicidal soaps. The reduction of microorganisms on skin using germicidal agents has been referred to as "degerming", and more recently, as "hygienic" hand disinfection, to distinguish it from "surgical" hand disinfection (Ayliffe *et al.*, 1978; Lilly and Lowbury, 1978; Ojajärvi *et al.*, 1977; Price, 1938a). A concept of "virtual" disinfection was suggested by Gardner (1948), if at least 99.9% of the microflora was removed or killed. The extent of skin disinfection depends on the method of washing. Pre-operative surgical scrubs are intended to remove both resident and transient microflora from the hands, whereas intermittent washing with germicidal soaps is expected to remove primarily the transient microflora (Ayliffe *et al.*,

1978; Lowbury *et al.*, 1964a; Ojajärvi, 1980).

Most researchers have attempted to evaluate skin germicides for surgeons and operating sites (Davies *et al.*, 1978; Lilly and Lowbury, 1971, 1974; Lowbury *et al.*, 1960, 1964b). Experiments in which hand washing was carried out with less thoroughness than surgical hand disinfection have been conducted on hospital staff (Bruun *et al.*, 1968; Ojajärvi, 1976, 1980; Ojajärvi *et al.*, 1977; Sprunt *et al.*, 1973). Some of these studies relate closely to needs for food hygiene, whereas studies specifically designed for food handling are limited (Brodie, 1965; Green, 1974; Horwood and Minch, 1951; Post and Balzer, 1963; Seligmann and Rosenbluth, 1975). A review of the literature on antimicrobial hand soaps for use in food service establishments was published by Crisley and Foter (1965). Since then, the use of hexachlorophene as a germicidal agent has been severely limited because of concerns for toxicity from dermal absorption (Pines, 1972).

Many comparative studies have been conducted on the efficacy of germicidal agents (Bruun *et al.*, 1968; Lilly and Lowbury, 1974; Lilly *et al.*, 1979; Lowbury and Lilly, 1973; Ojajärvi, 1976, 1980; Smylie *et al.*, 1973). A recurring problem is the methodology for testing product efficacy. There are no official methods for these agents comparable to the A.O.A.C. Use-Dilution method for sanitizer efficacy on inanimate surfaces (Association of Official Analytical Chemists, 1975). Multiple basin techniques to measure the

rate of mechanical removal of microorganisms from the skin have been established (Cade, 1952; Price, 1938a). Evaluation of skin germicides has been conducted on the natural skin microflora and by applying test microorganisms to the skin (Ayliffe *et al.*, 1978; Lowbury *et al.*, 1964a; Marples and Kligman, 1974; Story, 1952). Recently, Ojajärvi (1980) used contaminated gauze as a method of contaminating finger tips. Several skin sampling techniques have also been used, such as finger tip imprints onto agar plates containing germicide inactivators (Ayliffe *et al.*, 1975; Ojajärvi, 1980; Ojajärvi *et al.*, 1977; Smylie *et al.*, 1973; Sprunt *et al.*, 1973); rinsing techniques using rubber gloves (Aly and Maibach, 1979; Lowbury and Lilly, 1960), bowls (Ayliffe *et al.*, 1978; Hurst *et al.*, 1960; Lowbury *et al.*, 1960), or plastic bags (Salzman *et al.*, 1968; Sprunt *et al.*, 1973) and the rinsings plated for bacteriological analysis; adhesive tape (Wilson, 1970); and by moist swabbing of the skin surface, followed by agitation of the swab in broth for plating or streaking onto an agar plate (Story, 1952).

The object of this study was to determine the efficacy of various hand wash and hand dip treatments to reduce the number of microorganisms released from the hands after washing, and hence to reduce potential contamination of foods.

B. Materials and Methods

Two methods of microbiological sampling were followed: sampling by rinse solution (Salzman *et al.*, 1968; Sprunt *et al.*, 1973) in which the hands were immersed in 100 ml of sterile letheen broth (LB), and by imprints of finger tips (Ojajärvi *et al.*, 1977; Sprunt *et al.*, 1973) on letheen agar (LA) plates using a 5-s contact time for thumb and finger tips. The problem of confluent growth of colonies on finger imprint plates was avoided by streaking the inocula with a sterile glass hockey stick.

The experiment was based on a Latin Square design (Table 3.1). Two separate experiments were run simultaneously, each consisting of a 6 X 6 design. In each experiment, six agents (including a non-germicidal control soap) were used by each of six subjects (male and female volunteers) over a period of six weeks. The experiments were randomized for subjects and sequence in which agents were used. Each subject used the assigned agent once a day, for two successive 15-s washes, on four consecutive days of the week. For the remaining three days of the week, no treatment was applied so that any carry-over effect from one agent to another would be eliminated, as well as to allow the skin microflora to become re-established. A different agent was used by each subject, each week, according to a randomized procedure.

The two experiments allowed ten germicidal agents or washing procedures to be studied. All products were liquid,

Table 3.1. *Latin Square designs (before randomization) for germicidal hand wash experiments A and B, each containing six subjects, and six agents used for six weeks.*

Experiment A

Week	I	II	III	IV	V	VI
Subject	Agent					
1	A*	B	C	D	E	F
2	B	C	D	E	F	A
3	C	D	E	F	A	B
4	D	E	F	A	B	C
5	E	F	A	B	C	D
6	F	A	B	C	D	E

Experiment B

Week	I	II	III	IV	V	VI
Subject	Agent					
1	G*	H	I	J	K	L
2	H	I	J	K	L	G
3	I	J	K	L	G	H
4	J	K	L	G	H	I
5	K	L	G	H	I	J
6	L	G	H	I	J	K

* agents A and G = non-germicidal soap (control)

See Tables 3.3 and 3.4 for identification of product codes.

except agent F, which was a germicidal bar soap. The agents were grouped based on their use as *hand dip* (in which hands were placed in 1.5 *l* of freshly prepared germicidal solution) or *hand wash* (using 5 *ml* of liquid soap). The iodophor (0.75% available iodine) and 4% chlorhexidine gluconate hand wash agents were incorporated as reference agents commonly used in surgical practice.

In the first experiment, hand dip agents and a germicidal bar soap (F) were evaluated with a non-germicidal control soap. The agents included:

A. non-germicidal liquid hand soap;

B. non-germicidal liquid hand soap (15-s), followed by a 15-s hand dip in sodium hypochlorite solution containing 50 p.p.m. available chlorine;

C. sodium hypochlorite (50 p.p.m. available chlorine);

D. quaternary ammonium (QAC) solution containing 930 p.p.m. benzalkonium chloride;

E. iodophor solution with 25 p.p.m. available iodine; and

F. germicidal bar soap containing 1.0% trichlorocarbanilide (TCC).

In the second experiment, all the agents were used in the wash procedure. The agents included:

G. non-germicidal soap (same as A above);

H. iodophor "tamed iodine" scrub containing 0.75% titratable iodine (West Chemical Products Ltd., Montreal, Canada);

I. chlorhexidine gluconate (4%) liquid detergent (Hibitane®, Ayerst Laboratories, Montreal, Canada);

J. germicidal liquid soap containing 0.5% 2,4,4'-trichloro-2'-hydroxy diphenyl ether (Irgasan DP 300), diluted to 0.25% active ingredient at the use concentration;

K. antiseptic liquid hand soap (40%) containing 0.65% *para*-chloro-*meta*-xylenol (PCMX), diluted to 0.325%;

L. synthetic liquid hand soap containing 0.5% tribromosalicylanilide (TBS).

A standard hand washing procedure was established, and supervised throughout the study. Hands were moistened under running tap water, then washed for 15 s either by dipping in germicidal solution or by washing with hand wash agent poured onto the palm of the hand, or with the bar soap held in the hands. During this period, four different movements were each repeated five times: rubbing palms and fingers together, followed by the left palm over right dorsum, then right palm over left dorsum, and finally interlacing the fingers. The washing was carried out to include the hands up to the wrist. After precisely 15 s exposure, the hands were rinsed under running tap water until all of the feeling of soapiness had been removed (ca. 15 to 20 s). Microbiological sampling was done, and the process repeated with a second 15-s wash and microbiological sampling.

The two sampling techniques were carried out concurrently: hand rinse (X) and finger imprint (Y). In the

hand rinse technique, 100 *ml* of DIFCO letheen broth (LB) containing 35 *g* of 4 *mm* diam. sterile glass beads were placed in a plastic bag (28.5 X 12.5 X 7.5 *cm*, 1.25 *mil*, Polyrama Plastics Ltd., Edmonton, Canada) for use as the rinse solution. In the finger imprint technique, prepoured plates of DIFCO letheen agar (LA) were used. The hand to be sampled was placed in LB and rinsed in a standard manner by rubbing the glass beads over the palm of the hand 20 times. The hand was blotted on a sterile paper towel, then the finger tips were placed on a LA plate for 5 s. The hands were rinsed under running water to remove any residues of LB, as well as to wet the hands for the washing procedure. The second and third samplings were done after the first and second 15-s treatments, respectively.

Because of the experimental procedure, left and right hands had to be used interchangeably. An example of the sampling protocol is shown in Table 3.2. Sampling from the left or the right hand was randomly assigned for each subject on day 1 of each week, then alternated on following days. Initial samples (before washing) for both hand rinse and finger imprint techniques were always taken from the same hand, thereafter, hand rinse samples were taken from the other hand, and finger imprint samples were taken from the same hand as the initial sample (see Table 3.2).

Appropriate serial dilutions of the hand rinse samples in 99 *ml* of 0.1% sterile peptone water were surface streaked onto prepoured plates of DIFCO standard plate count agar

Table 3.2. *Example of left and right hand sampling protocol used for four successive days of a week.*

Day	Sampling times[1]	Sampling method	
		hand rinse	finger imprint
1	1	L[2]	L
	2	R	L
	3	R	L
2	1	R	R
	2	L	R
	3	L	R
3	1	L	L
	2	R	L
	3	R	L
4	1	R	R
	2	L	R
	3	L	R

[1] 1=before treatment,
2=after 15-s treatment,
3=after second 15-s treatment

[2] L=left hand, R=right hand

(SPC). Duplicate plates of each dilution were incubated at 35 C for 48 h. The finger imprint (LA) plates were immediately spread using a sterile glass "hockey stick" and incubated under the same conditions as described above.

A total of 3,591 cultures were isolated from the finger imprint plates for each treatment on days 1 and 3 of each week over the six week testing period. Colonies growing on the plates were selected based on the numbers and types of different bacterial colonies, similar to the procedure used for detecting coagulase-positive *Staphylococcus aureus* (Health Protection Branch, 1974). For colony types with an average count of less than five, all colonies were picked; for 5 - 25 colonies, five were picked at random; for 25 - 99 colonies, 8 were picked; and for >99 colonies, the square root of the total number were picked. Isolates were streaked onto SPC plates and incubated at 35 C for 24 h to check their purity. A single colony was picked and inoculated onto a nutrient agar slant and subjected to the following screening tests: Gram stain and microscopic morphology, catalase and oxidase tests. The cultures were subdivided according to the results of the four screening tests and subjected to appropriate identifying techniques based on the eighth edition of Bergey's Manual (Buchanan and Gibbons, 1974) and Holt's shorter version of Bergey's Manual (Holt, 1977). The differentiation of the Micrococcaceae was based on several additional references (Baird-Parker, 1974; Baird-Parker *et al.*, 1976; Kloos and Schleifer, 1975b;

Mitchell and Baird-Parker, 1967; Schleifer and Kloos, 1975; Whittenbury 1964). For the Enterobacteriaceae, Edwards and Ewing (1972), and for the pseudomonads, King *et al.* (1954) were used as additional bases for identification.

A range of standard strains of bacteria was used to check the identification procedures, including: *Staphylococcus aureus* ATCC 6538 (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland), *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus saprophyticus* U24423 (Alberta Laboratory of Public Health, Edmonton, Canada), *Aerococcus viridans* BC420 (Alberta Laboratory of Public Health), *Streptococcus faecalis* ATCC 7080, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 11229.

Statistical analysis of the data was carried out using a statistical package adapted to the Latin Square design (BMDP2V. Biomedical Computer Programs. P-series. 1979. University of California Press). Data were calculated as ratios based on the number of organisms released after the first and second 15-s treatments relative to the initial numbers released. For a normal distribution of the ratio data, log transformed ratio data were used in the analyses.

C. Results

Initial studies were carried out to verify the procedures. The protocol required interchangeable use of left and right hands. Samples were taken of each hand and

analyzed for differences in microbial load of the most and least frequently used hand. A Student t-test for paired data indicated no significant difference ($P>0.05$) in microorganisms released from each hand.

The effect of different rinse solutions for hand sampling on microbial counts was studied. The rinse solutions included distilled water, normal (0.85%) saline, 0.1% peptone water, 10% nutrient broth (NB) in distilled water or normal saline, and full strength NB. When microorganisms isolated from hands were suspended in these solutions, there was no significant change in the microbial counts during 2 h at 20 C.

The need for rinse solutions to inactivate the germcidal agents was also studied. The rinse solutions included 0.1% peptone water, 10% NB in distilled water, and full strength NB. Only NB gave an indication of inactivating the germicidal agents. Further study showed that the chlorhexidine and Irgasan DP 300 agents were not adequately inactivated by NB.

Residues of chlorhexidine and Irgasan DP 300 released from hands after the standard wash and rinse procedure were determined. It appeared that residues equivalent to between 0.01% and 0.001% of their use concentrations were released in the rinse solution. As a result, 0.1, 0.01, and 0.001% concentrations of the use dilution of these agents were added to NB and LB. NB failed to inactivate chlorhexidine at 0.001% and Irgasan DP 300 at 0.01% of their use

concentrations. In contrast, in LB there was slight inhibition of cells at 0.1% chlorhexidine, but not at 0.01%; while Irgasan DP 300 was inactivated in LB at all concentrations tested. LB and LA were therefore used as the rinse solution and growth medium for the hand rinse and finger imprint techniques, respectively.

The mean counts of the number of microorganisms released using the hand rinse or finger imprint sampling techniques, before and after washing with the various agents, were calculated for each agent used by six subjects for four consecutive days (24 observations per agent). These data and the percentage change in number of microorganisms released after the first and second 15-s washes are shown in Tables 3.3 and 3.4. Results for total hand sampling (hand rinse technique) generally gave counts of 10^3 per ml of LB rinse solution, representing about 10^5 organisms released from the hands, compared to the finger imprint technique that generally gave counts of 10^2 organisms on the LA plates. Correlation coefficients calculated between the two sampling methods ranged from 0.23 to 0.58, showing a weak linear relationship between the two methods.

From the data in Tables 3.3 and 3.4, it can be seen that the non-germicidal hand wash generally resulted in an increase in number of microorganisms released from the hands after the first 15-s wash, compared to a moderate decrease after the second 15-s wash. Some of the hand dip agents appeared to reduce the number of microorganisms released,

Table 3.3. *Mean count and percentage change in colony forming units released from hands after each of two successive 15-s treatments with HAND DIP agents using two sampling techniques.*

AGENTS	Initial	Hand rinse technique 1st wash mean count x10³ (percent)	2nd wash (percent)
A. control soap	9.0	10.5(117)	8.1 (90)
B. soap + hypochlorite (50 p.p.m.)	7.2	9.5(132)	6.2 (86)
C. hypochlorite (50 p.p.m.)	10.8	9.4 (87)	9.0 (83)
D. QAC (930 p.p.m.)	9.3	8.9 (96)	5.8 (62)
E. iodophor (25 p.p.m.)	7.0	8.2(117)	6.3 (90)
F. bar soap (1.0% TCC)	5.1	6.5(128)	5.9(116)
	Initial	Finger imprint technique 1st wash mean count x10³	2nd wash (percent)
A. control soap	9.7	13.6(140)	8.6 (89)
B. soap + hypochlorite (50 p.p.m.)	6.3	9.3(148)	8.8(139)
C. hypochlorite (50 p.p.m.)	11.8	5.2 (44)	5.8 (49)
D. QAC (930 p.p.m.)	7.7	4.0 (52)	1.7 (22)
E. iodophor (25 p.p.m.)	6.3	4.6 (73)	2.6 (41)
F. bar soap (1.0% TCC)	2.8	3.6(129)	3.4(121)

Table 3.4. *Mean count and percentage change in colony forming units released from hands after each of two successive 15-s treatments with HAND WASH agents using two sampling techniques.*

AGENTS	Initial	1st wash	2nd wash
	Hand rinse technique mean count x10³	(percent)	
G. control soap	8.9	10.4(117)	8.4 (94)
H. iodophor	13.3	2.4 (43)	1.7 (30)
I. chlorhexidine	3.7	2.2 (60)	1.8 (49)
J. Irgasan DP 300	6.8	8.2(121)	7.4(109)
K. PCMX	8.3	7.5 (90)	5.3 (64)
L. TBS	8.5	8.2 (97)	5.8 (68)
	Finger imprint technique		
	Initial	1st wash	2nd wash
	mean count x10³	(percent)	
G. control soap	8.1	6.7 (83)	10.5(130)
H. iodophor	6.4	2.1 (33)	0.6 (9)
I. chlorhexidine	4.8	0.6 (13)	0.2 (4)
J. Irgasan DP 300	9.8	8.3 (85)	6.6 (67)
K. PCMX[1]	11.5	8.9 (77)	10.4 (90)
L. TBS[2]	5.9	6.8(115)	9.1(154)

[1] *Para*-chloro-*meta*-xylenol
[2] tribromosalicylanilide

especially when compared by the finger imprint technique, but the bar soap containing 1% trichlorocarbanilide appeared to be ineffective. Of the hand wash agents, only chlorhexidine and iodophor (agents H and I) appeared to be effective in reducing the number of microorganisms released from the hands.

Analyses of variance for the Latin Square designs were carried out on the log transformed ratio data, representing the change in the number of microorganisms released after the two successive 15-s washes. In the hand dip experiment, using the hand rinse sampling technique, no significant difference ($P=0.59$) could be attributed to the germicidal agents. However, by the finger imprint sampling technique, agents had a significant effect ($P<0.001$). Further study of this effect using Duncan's multiple range test indicated that the difference could be attributed to the QAC dip after the first and second 15-s treatment, and the iodophor after the second treatment.

In the hand wash experiment, agents had a significant effect ($P<0.001$) by both sampling techniques. Results of the Duncan's multiple range test at the 95% confidence level are shown in Table 3.5. Using data for the hand rinse sampling technique, after the first 15-s exposure, the iodophor hand wash agent was significantly better than all agents except 4% chlorhexidine. However, after the second 15-s treatment, both iodophor and chlorhexidine agents gave a significant decrease in number of microorganisms released from the

Table 3.5. *Summary of Duncan's multiple range test (95% confidence level) for comparison between log ratio means for the hand wash agents.* [1] [2]

(i) Hand rinse technique (rank order of means)

After first 15-s wash	H	I	J	K	L	G
			———	———	———	———
		———	———			
	———	———				
After second 15-s wash	H	I	L	J	K	G
	———	———	———	———	———	———

(ii) Finger imprint technique (rank order of means)

After first 15-s wash	I	H	G	L	K	J
	———	———	———	———	———	———
After second 15-s wash	I	H	L	J	G	K
	–	–	———	———	———	———

[1] Hand wash agents:

G non-germicidal liquid hand wash (control),

H Iodophor (0.75% available iodine),

I 4% chlorhexidine gluconate (Hibitane),

J Irgasan DP 300 (0.25% use concentration),

K *para*-chloro-*meta*-xylenol soap (0.325% use concentration),

L tribromosalicylanilide soap (0.5% use concentration).

[2] Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

hands. Using data for the finger imprint sampling technique, both chlorhexidine and iodophor agents caused a significant decrease in microorganisms released from the hands, after both the first and second treatments. Analyses for a time sequence effect were not significant, indicating that there was no cumulative or persistence (substantive) effect for these agents with this protocol over the four-day testing period.

A total of 3,591 isolates were selected from finger imprint plates before and after the two 15-s treatments, on days 1 and 3 for each week of the study. The types of organisms isolated based on the four screening tests are shown in Table 3.6. There were five main groups (I-V) that could be identified. Group I, gram-positive coccus-shaped, catalase-positive, oxidase-negative organisms predominated. Group I organisms accounted for 85.3% of all isolates; 79.9% of the 1,935 isolates from plates of finger imprint samples before hand washing, and 91.5% of 1,656 isolates from plates after the two 15-s hand washes. The other groups (II-V) of microorganisms each represented less than 3.0% of the total isolates, but these groups of organisms were generally present in slightly greater concentration before than after washing with germicidal agents.

Since the gram-positive coccus-shaped, catalase-positive, oxidase-negative (group I) microorganisms were such a large group, a randomized sample of 264 (8.6%)of these isolates, representing each sub-group based on colony

Table 3.6. *Frequency of different screening test groups (gram stain, morphology, catalase and oxidase tests) of microorganisms isolated from finger imprint samples from hands.*

Group	Screening test[1]	Frequency					
		Total		Before wash		After wash	
		No.	%	No.	%	No.	%
I	1 1 1 2	3062	85.3	1547	79.9	1515	91.5
II	1 1 2 2	46	1.3	34	1.8	12	0.7
III	1 2 1 2	91	2.5	64	3.3	27	1.6
IV	2 2 1 1	38	1.1	28	1.4	10	0.6
V	2 2 1 2	60	1.7	43	2.2	17	1.0
Others		90	2.5	74	3.9	16	1.0
No growth		204	5.6	145	7.5	59	3.6

[1]Numerical codes refer, in sequence, to:
Gram stain 1=positive, 2=negative;
Cell morphology 1=coccus, 2=rod;
Catalase and Oxidase tests 1=positive, 2=negative.

size and appearance, was subjected to further identification. Based on the identification criteria: oxidative and fermentative reactions on glucose and mannitol, motility, novobiocin sensitivity and resistance, hydrogen peroxide formation and coagulase production, these isolates were identified as shown in Table 3.7. A total of 220 (83.3%) of these isolates were *S. epidermidis* and 44 (16.7%) were *Micrococcus* spp. No *S. aureus*, *S. saprophyticus* or *Aerococcus* spp. were detected among these isolates.

The 46 group II isolates were grown on 5% sheep blood agar plates and inoculated into nutrient broth for incubation at 45 C. The results indicated that these organisms were non-group D, gamma-hemolytic *Streptococcus* spp. The 91 group III isolates were checked for spore production and motility, and were identified as 50% *Bacillus* spp. and 50% *Corynebacterium* spp.

The group IV isolates were oxidase-positive, gram-negative rods. Using oxidative and fermentative reactions on glucose, motility and growth in nutrient broth at 42 C as differentiating tests, the 38 isolates were identified as *Moraxella* spp. (44%), *Flavobacterium* spp. (28%) and *Pseudomonas* spp. (28%). The group V isolates were oxidase-negative, gram-negative rods. They were also identified using oxidative and fermentative reactions on glucose and motility tests, and in the case of possible Enterobacteriaceae by the BBL Minitek technique (Becton Dickinson Canada, Mississauga, Ontario). These 60 isolates

Table 3.7. *Differentiation of* Micrococcaceae *(gram positive cocci, catalase-positive, oxidase-negative).* [1]

	Glucose		Mannitol		Novo-biocin[2]	Hyd. perox.	Coagul-ase
	Ox.	Ferm.	Ox.	Ferm.			
S. aureus	+	+	+	+	S	-	+
S. epidermidis	+	+	-	-	S	-	-
S. saprophyticus	+	weak	+	-	R	-	-
Micrococcus spp.	V[3]	-	V	-	V	-	-
Aerococcus spp.	+	+	V	-		+	-

[1] Based on Baird-Parker, 1974; Baird-Parker *et al.*, 1976; Holt, 1977; Schleifer and Kloos, 1975; and Whittenbury, 1964.
[2] S=sensitive to 0.6 ug novobiocin/ml; R=growth in the presence of 1.6 ug novobiocin/ml (Kloos and Schleifer, 1975a).
[3] V=variable positive or negative reaction.

were identified as *Acinetobacter* spp. (70%), *Pseudomonas* spp. (15%), and Enterobacteriaceae (15%) which included one *Escherichia coli* isolate and four *Klebsiella pneumoniae* isolates.

The incidence of isolates that failed to grow (Table 3.6) at time of identification was attributed to the length of time that they were stored on nutrient agar under refrigeration. However, this did not affect the results for the screening tests because they were carried out at the time that the isolate was originally taken from the plating medium.

D. Discussion

This evaluation of germicidal hand dips and hand washes for use by food handlers was based on the ability of the agents to reduce the number of bacteria released from the hands after washing, hence reducing the potential for food contamination with bacteria from this source. The two methods for assessing the bacteria released could have different practical implications. Although the hand rinse sampling technique is generally considered to be more reliable, it might be argued that in many cases the finger tips are more significant to food contamination than the complete hand. The finger imprint method is often used for field studies because it is time saving and conducive to collaboration of workers (Smylie *et al.*, 1973), however it is generally considered to be less reliable than other

sampling techniques (Ayliffe *et al.*, 1975; Ojajärvi, 1980; Ojajärvi *et al.*, 1977; Sprunt *et al.*, 1973). In our studies, the finger imprint data gave more favorable efficacy data than the hand rinse data.

It has been suggested that germicidal hand wash agents should be evaluated by in-use tests, which closely resemble practical conditions (Ojajärvi, 1976). An important aspect of practical conditions for this study was the time of exposure to germicidal agents. Short exposure times have been reported to range from 10 to 30 s (Hurst *et al.*, 1960). Many hospital oriented studies have used 30 s to 2 min exposure times, some have used 15 s (Ojajärvi, 1980). For this study, two successive 15-s washes were used, because a 15-s wash was considered more likely to be achieved in practice. The results indicated that the second 15-s wash, included to indicate whether a total 30 s wash should be recommended, did not have a marked influence on the efficacy of the germicidal agents.

The correlation between the results for the two testing techniques in this study was relatively poor, representing a weak linear relationship between the two tests. Other workers (Ayliffe *et al.*, 1975) reported "fairly good correlation" between finger imprint and hand rinse techniques, but the correlation coefficients were not given. From this study it appears that the two sampling techniques are measuring different microbial parameters. However, there are possible differences in exposure times of the different

areas of the hand in the hand wash experiment, as opposed to the hand dip experiment. In hand washing, a 5 *ml* aliquot of germicidal agent was applied to the palm of the hand, and timing was begun. The agent was then spread progressively over the hand, commencing with the finger tips. Furthermore, the hand wash technique only used four different movements within the 15-s wash time, and represented a practical type of hand wash that might be expected of food handlers. Other procedures have been more exhaustive, including rotational rubbing of the thumb and fingers in the palm of the hand (Ayliffe *et al.*, 1978). Nonetheless, similar opinions of the value of agents have been reported by different workers using different techniques (Lowbury and Lilly, 1973).

The results were initially analyzed on the basis of the log number of bacteria released from the hands. The significant differences between subjects and the same individual from day to day made such analyses meaningless. Similar results have been reported by other workers (Hurst *et al.*, 1960) and hence analyses were based on the 15 s and total of two treatments (30 s) "reduction factor" as used by Lilly *et al.* (1979) and described by Rotter *et al.* (1974).

Some agents, notably the non-germicidal soap, resulted in higher counts after the first 15-s wash than before washing. Similar increases in count have been reported for non-germicidal soaps (Ayliffe *et al.*, 1978; Brodie, 1965; Lilly *et al.*, 1979), while others reported moderate decreases in counts after one and six applications (Lilly

and Lowbury, 1971, 1974). Bar soap has been cited as effective for control of transient microorganisms (Lowbury *et al.*, 1964a), so that little added value may be expected from germicidal hand washes for 30 s. In our studies some agents produced a significant reduction in the number of microorganisms released from the hands after the first and second 15-s exposures.

The hand dips used in the first experiment were generally unsatisfactory, because they failed to give a reduction in number of bacteria released compared to non-germicidal soap. The only exception to this was the QAC hand dip. The positive electrical charge of the QAC on the skin is considered to account for the retention of bacteria on the cutaneous surface (Blank and Coolidge, 1950). The use of QAC (1.2% benzalkonium chloride) was discontinued in another study (Bruun *et al.*, 1968), because of skin irritation and poor reduction of microbial counts. The iodophor hand dip with 25 p.p.m. available iodine gave poor reduction of microbial counts compared to the iodophor hand wash containing 0.75% titratable iodine.

The bar soap containing 1% trichlorocarbanilide (TCC) was included in these studies because it was the only form in which this germicide was commercially available. Results with this agent were disappointing, resulting in overall increases in number of bacteria released from the hands. A similar result was observed (Bibel, 1977), but a change in flora from *S. epidermidis* to *Acinetobacter* and *Micrococcus*

was reported. Successful studies have been reported with 1% and 2% concentrations of TCC (Roman *et al.*, 1958; Wilson, 1970). These concentrations of TCC were reported to give results similar to those for the same concentrations of hexachlorophene (Hurst *et al.*, 1960).

Many studies of germicidal agents have been designed to determine immediate and persistent (substantive) efficacy of the agents (Hurst *et al.*, 1960; Lilly and Lowbury, 1971, 1974; Lowbury and Lilly, 1973; Ojajärvi, 1976) by testing for bacterial reduction after one wash and after six successive washes, usually over a two day test period. In this study, the "immediate" efficacy was studied. However, the occurrence of a substantive effect over the four day test period was checked, but no substantivity was observed with any of the agents. Hexachlorophene used exclusively (Cade, 1952; Lowbury and Lilly, 1960), and other agents including 4% chlorhexidine gluconate (Lilly and Lowbury, 1971; Lowbury and Lilly, 1973; Ojajärvi, 1976), and Irgasan DP 300 (Lilly and Lowbury, 1974) have been reported to give cumulative effects.

Hexachlorophene was excluded from our studies because it's use has been discontinued by many manufacturers, and Canadian regulations limit it's use to 0.75% without medical prescription (National Health and Welfare, 1980). Chlorhexidine was included as a medical reference agent, and the iodophor was marketed both for medical use and as a germicidal hand wash agent for food handlers. These were the

only products that resulted in a significant decrease in the number of bacteria released from hands in the hand wash experiment. Other researchers have reported even greater reductions in microbial contamination of hands with one application of 4% chlorhexidine gluconate (Ayliffe *et al.*, 1975; Lilly *et al.*, 1979; Lowbury and Lilly, 1973; Ojajärvi, 1980; Smylie *et al.*, 1973) than we observed, however some marked differences have been noted between different types of chlorhexidine preparations (Lilly and Lowbury, 1971; Lowbury and Lilly, 1973; Lowbury *et al.*, 1964b; Ojajärvi, 1976). Iodophor was reported to give 50 to 70% reduction of microflora on the hands (Lowbury and Lilly, 1973; Ojajärvi, 1976) with poor cumulative action (Lowbury *et al.*, 1964b), yet up to 90% reduction after 6 successive treatments (Lowbury and Lilly, 1973). In our studies, the iodophor hand wash gave 60 to 70% reduction of the bacteria released after 15-s exposure by the two techniques. None of these agents achieved levels of "virtual disinfection", i.e. 99.9% reduction in the number of bacteria released from the hands.

The other agents, including the *para*-chloro-*meta*-xylenol (PCMX), Irgasan DP 300 and tribromosalicylanilide gave poor germicidal results in this experiment. PCMX is a well known antiseptic agent, but there have been conflicting reports about its efficacy (Dankert and Schut, 1976), especially related to formulation and in-use concentration. Irgasan DP 300 at a use concentration of 0.25% in this study gave poor results. Use of 0.6% of this agent was reported to

be poor in reducing number of bacteria on the hands, and not much better than ordinary soaps (Ojajärvi, 1976), while 0.75% use concentration gave 20.9% reduction after one application and 56.2% after six applications (Lilly and Lowbury, 1974). A concentration of 2% Irgasan DP 300 was reported to be necessary to achieve comparable results with those observed using 3% hexachlorophene or 4% chlorhexidine gluconate (Lilly and Lowbury, 1974).

The predominating microorganisms isolated and identified in these studies were *S. epidermidis* and *Micrococcus* spp., typical of resident bacteria on skin, that are harmless commensals (Evans *et al.*, 1950; Noble and Somerville, 1974; Somerville, 1969a,b). Anaerobic *Corynebacterium acnes*, which has been shown to predominate on skin of some individuals (Evans *et al.*, 1950), would not be detected by our methods. The subjects selected for these studies were laboratory workers and food handlers, however the food handlers were not working in a commercial food operation, and did not have high levels of transient bacteria such as described by Seligmann and Rosenbluth (1975). The transient-type bacteria identified among the isolates were reduced after washing, in agreement with other reports (Ayliffe *et al.*, 1975; Sprunt *et al.*, 1973). The incidence of enteric-type organisms (coliforms and faecal *E. coli*) on hands has been associated with meat and food handling (Brodie, 1965; Seligmann and Rosenbluth, 1975) and on hands of nurses in nurseries (Sprunt *et al.*, 1973), but

they are readily removed by hand washing, even with soap and water (Brodie, 1965).

From the standpoint of the reduction of microorganisms released from the hands, many of the germicidal agents tested in this study were no better than the non-germicidal soap. Notable exceptions to this were the hand wash agents containing chlorhexidine gluconate and the iodophor. The former is not a product that would find ready acceptance in the food industry in its present form, however the latter is marketed for both medical and food industry use. The microbiological studies indicated that most bacteria identified were resident-type rather than transient-type skin microorganisms. It is generally considered that transients may not act like resident (normal) skin flora in the washing process, and that transient microorganisms represent the major concern in cross-contamination (Ojajärvi, 1980). It has been suggested that soap and water might be adequate for general hand washing, and that germicidal agents would only be required for aseptic procedures (Ayliffe *et al.*, 1975). Our results, based on the desirability of reducing contamination of foods with microorganisms from hands, did not support this attitude. However, a study specifically related to the control of transient-type bacteria on hands would add to this information on the efficacy of germicidal hand wash agents for hygienic hand disinfection.

IV. IMMEDIATE AND RESIDUAL (SUBSTANTIVE) EFFICACY OF GERMICIDAL HAND WASH AGENTS

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A. Introduction

The retention of germicidal residues on the skin after washing is referred to as residual effect or substantivity. This characteristic is attributed to the physicochemical binding of the agent to the uppermost horny layer of the skin (Marples and Kligman, 1974). Substantivity gives the advantage of reducing the skin microflora and preventing its colonization with pathogenic microorganisms. This effect may last for a few days depending on the type of germicide and the frequency and exclusivity of use (Duncan *et al.*, 1969; Hall, 1980; Ojajärvi *et al.*, 1977).

Hexachlorophene was one of the most common germicides in use. It relied heavily on substantivity for its germicidal efficacy (Lilly and Lowbury, 1974; Van der Hoeven and Hinton, 1968). However, the use of hexachlorophene has decreased because of its toxicological implications, and regulations restricting its use concentration to 0.75% without medical prescription (National Health and Welfare,

1980; Pines, 1972). Alternative agents have been introduced to replace hexachlorophene, such as, chlorhexidine, which has a dramatic, immediate effect on the resident and transient microflora of the skin (Ayliffe *et al.*, 1975; Hall, 1980; Lilly and Lowbury, 1974; Lilly *et al.*, 1979; Lowbury and Lilly, 1973), and an excellent residual effect (Lilly and Lowbury, 1974; Lowbury and Lilly, 1973; Ojajärvi, 1976).

Other germicidal agents with a reported residual effect include various phenolic derivatives, such as chloroxylenol and 5-chloro-2-(2,4-dichlorophenoxy)phenol (Triclosan or Irgasan DP 300). Chloroxylenol formulated with EDTA (ethylenediaminetetraacetic acid) was shown to be highly bactericidal for skin microflora, and the effect persisted for a minimum of 2 hours (Dankert and Schut, 1976). Irgasan DP 300 used at 2% concentration was shown to have a residual effect (Lilly and Lowbury, 1974), but at 0.6 and 0.75% use concentrations this effect was poor (Ayliffe *et al.*, 1975; Lilly and Lowbury, 1974; Ojajärvi, 1976).

Iodophor is widely used as a germicide for disinfection of the skin of hands and operation sites (Joress, 1962; Lowbury and Lilly, 1973; Smylie *et al.*, 1973). It is generally used at 0.75% available iodine (Lowbury and Lilly, 1973; Ojajärvi, 1976), and it is one of the most effective germicidal agents for removal of *Staphylococcus aureus* from the skin (Lowbury *et al.*, 1964a). Iodophor markedly reduces the number of bacteria on hands after six washes over two

successive days (Lowbury and Lilly, 1973), however it does not have a residual effect (Ojajärvi, 1976; Peterson *et al.*, 1978; Smylie *et al.*, 1973; Van der Hoeven and Hinton, 1968).

In an earlier paper (Sheena and Stiles, 1982), we reported the efficacy of commercial hand washes and hand washing regimes used by food handlers. Only the iodophor (0.75% available iodine) and 4% chlorhexidine gluconate gave a significant decrease in the number of microorganisms released from hands after washing for short exposure times. The purpose of this study was to determine the immediate and residual (substantive) effect of various germicidal agents in commercial preparations, in particular, products containing Irgasan DP 300 or *para*-chloro-*meta*-xylenol (PCMX).

B. Materials and Methods

The study consisted of two separate experiments, both were Latin Square designs, one a 4 x 4, the other a 6 x 6 design. The agents in Experiment I (4 x 4 design) included: (A) chlorhexidine gluconate (4%) liquid detergent (Hibitane®, Ayerst Laboratories, Montreal, Canada) as a reference agent; and three products containing 2,4,4'-trichloro-2'-hydroxy diphenyl ether (Irgasan DP 300, Ciba-Geigy Ltd., Switzerland) as the active ingredient, (B) an antibacterial gel skin cleanser containing 0.3% Irgasan DP 300, (C) germicidal liquid soap containing 0.5% Irgasan DP 300, and (D) agent C diluted to 0.25% Irgasan DP 300. The

agents in Experiment II (6 x 6 design) included: (A') the chlorhexidine gluconate (4%) reference; (E) an iodophor hand wash containing 0.005% available iodine and (F) iodophor ("Tamed Iodine" Scrub, West Chemical Products Ltd., Montreal, Canada) containing 0.75% available iodine; and three products containing *para*-chloro-*meta*-xylenol (PCMX) as the active ingredient, (G) a gel containing 3% PCMX, (H) antiseptic liquid hand soap (40%) containing 0.65% PCMX and (I) agent H diluted to 0.325% PCMX. Products D and I corresponded to products J and K in our earlier study (Sheena and Stiles, 1982).

Washing procedures in both experiments were identical and followed the method previously described (Sheena and Stiles, 1982), with two successive 15-s exposures and the finger tip sampling technique, using separate plates for each hand. Finger tip imprints were made onto letheen agar (Difco) plates, and the inocula were spread using a sterile, glass "hockey stick". All plates were incubated at 35 C for 48 h. Hands were washed three times per day on two successive days, as performed in studies by Ayliffe et al. (1975) Lilly and Lowbury (1974) and Lowbury and Lilly (1973). No testing was carried out for the following five days, to allow any residual effect to be dissipated, and for the skin microflora to become re-established.

Samples were collected before and after the first and sixth washes, before each intermediate wash, and finally, without further treatment, the morning after the final wash

had been completed, as illustrated in Table 4.1. Subjects were instructed to avoid the use of any other germicidal hand wash agents during the course of the experiments. They were permitted to use non-germicidal soaps, and no control was exercised over the contamination of hands.

The data were calculated as ratios of the number of organisms released from the finger tips after washing, compared to the number released before washing. The data were analyzed in three different ways using $\log_{10}$ transformed ratios in a statistical computer package for Latin Square designs (BMDP2V, Biomedical Computer Programs, P-Series, 1979, University of California Press): (i) immediate reduction effect, based on the change in number of bacteria released during the first treatment, Y_1/Y_0 and Y_2/Y_0; (ii) the reduction effect after six successive treatments over two days, Y_6/Y_7 and Y_9/Y_7; and (iii) the day to day residual (cumulative) effect based on Y_5/Y_0 and Y_{10}/Y_0.

C. Results

The mean number of microorganisms and the percentage change as a result of the germicidal hand wash treatments are shown in Table 4.2. The 4% chlorhexidine gluconate and iodophor (0.75% available iodine) hand washes gave a marked reduction in the number of microorganisms released from the finger tips. In most other cases, the washing procedure resulted in an increase in the number of microorganisms

Table 4.1. *Washing and sampling protocol to determine the residual efficacy of germicidal hand wash agents.*

Day	(time)	Wash sequence	Treatment	Sampling
1	(8 am)	1st	No treatment	Y_0 - initial count
			1st 15-s wash	Y_1 - after 1st wash
			2nd 15-s wash	Y_2 - after 2nd wash
	(11 am)	2nd	2x15-s washes	Y_3 - before washes
	(2 pm)	3rd	2x15-s washes	Y_4 - before washes
2	(8 am)	4th	2x15-s washes	Y_5 - before washes
	(11 am)	5th	2x15-s washes	Y_6 - before washes
	(2 pm)	6th	Before treatment	Y_7 - before washes
			1st 15-s wash	Y_8 - after 1st wash
			2nd 15-s wash	Y_9 - after 2nd wash
3	(8 am)		No treatment	Y_{10} - final sampling

Table 4.2. *Percentage mean change in colony forming units released from finger tips after one and six washing sequences with germicidal hand wash agents.* [1]

Agent	First wash sequence			Sixth wash sequence		
	Init	1st 15-s	2nd 15-s	Init	1st 15-s	2nd 15-s
	Number of microorganisms x10[1]					
Experiment I.						
A	84.4	8.6 (26)	1.6 (2)	7.9	0.3 (18)	0.1 (8)
B	17.0	16.2 (90)	16.1 (89)	47.6	64.1(153)	60.4(151)
C	24.5	27.0(144)	43.6(251)	25.1	16.9 (70)	17.4 (72)
D	16.8	23.6(173)	27.4(219)	17.1	17.9(278)	21.2(332)
Experiment II.						
A'	13.8	5.9 (61)	1.4 (13)	3.1	0.3 (14)	0.2 (8)
E	11.7	19.9(157)	20.8(179)	13.0	7.7 (45)	9.6 (53)
F	9.4	4.8 (51)	3.5 (37)	9.0	4.8 (40)	4.1 (32)
G	14.4	23.4(140)	24.3(148)	10.1	13.1(134)	14.4(145)
H	13.4	20.4(198)	21.7(193)	11.5	10.8 (90)	19.3(122)
I	15.6	20.0(161)	21.8(206)	24.4	29.5(136)	31.3(139)

[1] Hand wash agents:

A and A' 4% chlorhexidine gluconate (Hibitane);

B,C,D Irgasan DP 300, 0.3% gel, 0.5% and 0.25% hand washes;

E and F iodophor products, 0.005% and 0.75% available iodine;

G,H,I *para*-chloro-*meta*-xylenol (PCMX) 3% gel, 0.65% and 0.325% hand washes.

[2] Mean counts and mean percentage survivors calculated from individual changes in count for each subject after the first and sixth wash sequences:

First wash sequence compared to first sampling, before treatment (Y_0);

Sixth wash sequence compared to sampling before sixth wash (Y_7).

released, except for the low concentration iodophor (0.005% available iodine), which caused a reduction in number of microorganisms released in the sixth washing sequence. The results of the analyses of variance indicated a highly significant effect attributable to germicidal agents ($P<0.01$). The results for the Duncan's multiple range tests for these data are shown in Tables 4.3 and 4.4. The analyses confirmed that the 4% chlorhexidine gluconate and iodophor (0.75% available iodine) products resulted in a significant reduction in the number of microorganisms released from the finger tips, compared to other agents. The iodophor wash (0.005% available iodine) was not significantly better than the PCMX agents.

The day to day residual or substantive effect is indicated by the initial number of microorganisms released from the hands, prior to treatment with the germicidal agents. This was determined initially (Y_0), before each subsequent treatment (Y_3 to Y_7) and on the third day, the morning after the sixth treatment (Y_{10}). The mean percentage number of microorganisms released from finger tips before each washing, compared to the initial count (Y_0), are shown in Table 4.5. The only product that gave a residual effect was 4% chlorhexidine gluconate. The initial number of microorganisms released from the finger tips was always less than the initial number released (Y_0), i.e. before the first treatment. The iodophor (0.75% available iodine) gave slightly reduced initial counts. The other agents failed to

Table 4.3. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means for the initial (first) hand wash sequence (Immediate Efficacy).* [1] [2]

(i) After 1st 15-s wash

Experiment I: A B C D

Experiment II: A' F G I E H

(ii) After 2nd 15-s wash

Experiment I: A B C D

Experiment II: A' F G H E I

[1]Explanation of product codes given in Table 4.2.

[2]Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

Table 4.4. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means for the final (sixth) hand wash sequence.* [1] [2]

(i) After 1st 15-s wash

Experiment I: A C B D

Experiment II: A' F E H G I

(ii) After 2nd 15-s wash

Experiment I: A C B D

Experiment II: A' F E H G I

[1] Explanation of product codes given in Table 4.2.

[2] Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

Table 4.5. *Percentage mean change in colony forming units released from finger tips before each hand wash treatment and the day after the final treatment (Residual Efficacy).* [1]

Treatment: Agent	2nd Y_3/Y_0	3rd Y_4/Y_0	4th Y_5/Y_0	5th Y_6/Y_0	6th Y_7/Y_0	Next day Y_{10}/Y_0
	Percent microorganisms released before each treatment, compared to initial count (Y_0).					
Experiment I.						
A	36	43	38	14	10	20
B	133	96	189	161	252	170
C	245	199	112	74	205	164
D	156	203	164	87	94	304
Experiment II.						
A'	89	73	63	45	35	74
E	108	119	134	132	132	138
F	73	94	84	84	92	108
G	179	142	151	144	144	115
H	227	172	176	238	151	193
I	131	141	141	168	131	144

[1]See Table 4.2 for agent codes.

achieve a general reduction in in the number of organisms released from the finger tips.

Analyses of variance were done on the $\log_{10}$ transformed ratio data for 24 h (Y_8/Y_0)and 48 h (Y_{10}/ Y_0) after treatment was initiated. Levels of significance were $P<0.05$ for Experiment I, comparing Irgasan DP 300 products to 4% chlorhexidine gluconate; and $P=0.001$ for Experiment II, comparing PCMX and iodophor products to 4% chlorhexidine gluconate. Differences among the means were not as significant as might be expected. Duncan's multiple range test for differences among treatment means are shown in Table 4.6. In Experiment I, there were no significantly different products at 24 h, and only 4% chlorhexidine gluconate and 0.25% Irgasan DP 300 differed significantly at 48 h. In Experiment II, 4% chlorhexidine gluconate gave a significant reduction in number of microorganisms released compared to the low concentration (0.325 and 0.65%) PCMX products, for both the 24 and 48 h tests. The results observed for 3% PCMX gel and the iodophor products were not significantly different from those for 4% chlorhexidine gluconate.

D. Discussion

This study enabled both the short- and longer-term (residual) efficacy of these germicidal hand wash agents to be assessed. The results for the immediate effect were in agreement with the results of our previous study (Sheena and

Table 4.6. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means 24 and 48 h after the initial wash sequence (Residual Efficacy).* [1] [2]

(i) After 24 h (before 4th wash Y_s/Y_o)

Experiment I: A C D B

Experiment II: A' F G E I H

(ii) After 48 h (morning after sixth treatment Y_{1o}/Y_o)

Experiment I: A C B D

Experiment II: A' G F E I H

[1] Explanation of product codes given in Table 4.2.

[2] Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

Stiles, 1982), in which only 4% chlorhexidine gluconate and iodophor containing 0.75% available iodine gave a significant reduction in number of microorganisms released from finger tips after washing for short exposure time.

In this study, the higher concentrations of the Irgasan DP 300 and PCMX products and the gel based formulations failed to give significant reductions in number of micro-organisms released from the finger tips. This indicates that the poor results that we reported for Irgasan DP 300 and PCMX, under our testing conditions (Sheena and Stiles, 1982), were not attributable to the lower use concentrations selected, and were probably not a problem with formulation.

The experimental protocol involving six hand wash treatments over two successive days has been used by other researchers (Ayliffe *et al.*, 1975; Lilly and Lowbury, 1974; Lowbury and Lilly, 1973) to assess the efficacy of repeated use of germicidal hand wash agents. For chlorhexidine gluconate a residual effect was observed, which agreed with other reports (Ayliffe *et al.*, 1975; Lilly and Lowbury, 1974; Lowbury and Lilly, 1973; Smylie *et al.*, 1973). The iodophor (0.75% available iodine) reduced the number of microorganisms released from hands, but the effect was not significantly different, and a residual effect was not indicated. The low concentration iodophor (0.005% available iodine) deserves further attention. Although it did not result in a significant reduction in number of micro-organisms released from the hands on initial usage, after

the sixth use a significant effect was observed. For the Irgasan DP 300 and PCMX products, no residual effect was noted. However, literature references indicated a residual effect for this agent under various conditions of use and concentration (Bodey and Rosenbaum, 1973; Dankert and Schut, 1976; Lilly and Lowbury, 1974; Russell and Furr, 1977).

The only agents that could be considered effective in reducing the number of microorganisms released from finger tips, under these conditions of hygienic hand disinfection using short exposure times, were the 4% chlorhexidine gluconate and iodophor (0.75% available iodine) products. The low concentration iodophor (0.005% available iodine) showed some promise, and warrants further study, because of the practical disadvantages of higher concentration iodophors, namely color and odor. This study indicated that alternative agents for hand washing with short exposure times are more likely to be found among the iodophor and chlorhexidine gluconate products, than the Irgasan DP 300 or PCMX products.

V. EFFICACY OF GERMICIDAL HAND WASH AGENTS AGAINST TRANSIENT BACTERIA INOCULATED ONTO HANDS

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A. Introduction

Earlier studies by Sheena and Stiles (1982, 1983a) assessed the efficacy of a range of germicidal hand wash agents against the total microflora occurring naturally on hands. The division of the skin microflora into transient and resident microorganisms has long been accepted (Price, 1938a). Transient microflora are those organisms acquired from the surroundings, which are superficially located on the skin, generally do not colonize the skin to become part of the resident microflora, and are readily removed by washing (Lowbury *et al.*, 1960, 1964a; Seligmann and Rosenbluth, 1975). Aerobic isolates identified after germicidal hand washing (Sheena and Stiles, 1982) revealed 85% *Staphylococcus epidermidis* and *Micrococcus* spp., indicating that the principal survivors after washing were organisms typically associated with the resident microflora of skin (Roskey and Hamdy, 1972; Seligmann and Rosenbluth, 1975).

The method of application of transient microorganisms to the skin (Lilly and Lowbury, 1978; Ojajärvi, 1980) and the species or strains of test organisms selected (Ayliffe *et al.*, 1978) play an important role in efficacy testing. Koller et al. (1978) observed differences in level of contamination between finger tip and hand immersion contaminating techniques, but no significant difference in the efficacy of hand disinfection attributable to the method of contamination. In earlier studies, the transient microorganisms were spread on the skin (Aly and Maibach, 1980; Dineen, 1978; Gardner and Seddon, 1946; Lowbury *et al.*, 1964a; Story, 1952), more recently, the natural work environment, has been used to contaminate skin with a transient microflora (Ojajärvi, 1980; Sprunt *et al.*, 1973). This natural environment approach was used in this study with meat as the suspending agent.

Most studies have used germicidal products intended for medical use, including 70% ethyl alcohol, 70% isopropanol, alcoholic preparations of chlorhexidine, 4% chlorhexidine gluconate liquid detergent, 0.75% povidone iodine, 2% Irgasan DP 300 and 3% hexachlorophene (Aly and Maibach, 1980; Ayliffe *et al.*, 1978; Lilly and Lowbury, 1978; Lowbury *et al.*, 1964a; Ojajärvi, 1980). Information on the use of these agents in food handling, using short exposure times, is scant. Crisley and Foter (1965) reviewed antibacterial soaps for hand washing in food service establishments. They concluded that hand washing with non-germicidal soap was

required to prevent transmission of possible pathogens from hands to foods during handling and preparation. Frequent hand washing by food handlers is considered mandatory to maintain hygienic conditions (Davis *et al.*, 1969; Pether and Gilbert, 1971; Seligmann and Rosenbluth, 1975).

The object of this study was to evaluate and compare the efficacy of germicidal hand wash agents for control of transient microorganisms inoculated onto hands from meat.

B. Materials and Methods

Two separate experiments were conducted, a 7 x 7 and a 5 x 5 Latin Square design, involving the exposure of each subject to each agent included in the experiment. The sequence in which agents were used by subjects was randomly assigned by a specified procedure (Myers, 1972). Each subject was exposed to all of the agents over the period of the experiment. Subjects used the assigned agent on two occasions, one 15-s exposure in the morning, and two successive 15-s exposures in the afternoon. The hand washing procedure was detailed in our previous study (Sheena and Stiles, 1982). There were two testing days per week (Monday and Thursday), so that two different products were tested on each subject, each week.

In the first experiment, seven agents were tested (A) non-germicidal liquid hand soap; (B) chlorhexidine gluconate (4%) liquid detergent (Hibitane®, Ayerst Laboratories, Montreal, Canada) as a positive control; (C) an

antibacterial gel skin cleanser containing 0.3% 2,4,4'-trichloro-2'-hydroxy diphenyl ether (Irgasan DP 300); (D) germicidal liquid soap diluted to 0.25% Irgasan DP 300; (E) antiseptic liquid hand soap containing 0.65% *para*-chloro-*meta*-xylenol (PCMX), diluted to 0.325% active ingredient at the use concentration; (F) iodophor ("Tamed Iodine" Scrub®) containing 0.75% available iodine; (G) germicidal bar soap containing 1.0% trichlorocarbanilide (TCC).

In the second experiment, five agents were tested: (A') non-germicidal liquid hand soap (same as "A" above); (H) iodophor hand wash containing 0.005% available iodine; and three hand dips consisting of (I) iodophor solution containing 25 ppm available iodine, (J) sodium hypochlorite solution containing 50 ppm available chlorine, and (K) quaternary ammonium (QAC) solution containing 930 ppm benzalkonium chloride. Hand wash agents were used in 5 ml amounts, the hand dip solutions were freshly prepared in 1.5 l deionized water.

Two bacterial strains were isolated from ground beef for use in this study: *Escherichia coli* and *Pseudomonas fluorescens*. The *E. coli* isolate was identified and confirmed by comparison with a standard strain of *E. coli* from the American Type Culture Collection (ATCC) strain #1229, and a strain (1840) previously isolated from ground beef (M.E.S.). The *P. fluorescens* isolate was similarly compared to a strain of *P. fluorescens* from the National

Collection of Type Cultures (NCTC) strain 10038 and a strain (R639) from the Alberta Laboratory for Public Health (Edmonton, Canada). Cultures were carried in nutrient broth. *E. coli* was incubated at 35°C for 18 h, and *P. fluorescens* was incubated at 20°C for 30 h for use in the experiments. Final concentrations for *E. coli* averaged 5 x 10^8 c.f.u./ml, and *P. fluorescens* averaged 1.1 x 10^8 c.f.u./ml.

A ground beef inoculum was prepared to inoculate the test organisms onto subjects' hands. Freshly prepared ground beef from a local retail store was inoculated with the *E. coli* and *P. fluorescens* test cultures, to give counts of 10^6 and 10^7 c.f.u./g of ground beef, respectively. This necessitated a 10^{-1} dilution of the *E. coli* culture in 0.1% peptone water, while the *P. fluorescens* culture was inoculated without dilution. The ground beef was checked each day to determine its microbiological quality, including total aerobic plate count, coliform, *E. coli*, *P. fluorescens* and total "gram positive cocci" counts. The levels of *E. coli* and *P. fluorescens* inocula in the ground beef were also determined. The inoculated ground beef was dispensed in two 50 g amounts in separate petri dishes to use as inocula for the fingertips.

To reduce the microbial load on the hands, in preparation for the experiment, subjects' rinsed their hands with 5 ml of 95% ethanol containing 1% glycerol, and the hands were rubbed together until they were dry (Ayliffe *et al.*, 1975; Ojajärvi, 1980). The finger and thumb tips were

pushed into and held in the ground beef inoculum for 5 s, and the inoculum was distributed over the hands by rubbing up to the wrists, until the hands were dry. The hands were washed on two separate occasions with the same agent (morning and afternoon wash). One of the hands was randomly selected for sampling for the initial count (X_0), by rinsing in 100 ml letheen broth (LB, Difco) in a plastic bag (28.5 x 12.5 x 7.5 cm, 25 mil, Polyrama Plastics Ltd., Edmonton, Canada) using the standard hand rinse method described in our previous study (Sheena and Stiles, 1982). Hand washing and dipping were also done according to the procedures previously described (Sheena and Stiles, 1982). The sample for the first 15-s wash (X_1) was taken at the morning wash. The sample for the two successive 15-s washes (X_2) was taken at the afternoon wash, and compared with the initial (X_0) count for the afternoon testing period. Subjects rinsed their hands with the glycerol in ethanol solution after the sampling procedure had been completed.

Bacteriological testing of the hand rinse samples was done by plating in duplicate onto the following Difco media: standard plate count agar (SPC), violet red bile agar (VRBA), Pseudomonas agar F (PAF) and Baird-Parker medium (B-P). Two sets of VRBA plates were prepared for comparison of the differential incubation temperatures. After washing hands with germicidal agents, such as chlorhexidine gluconate (4%) liquid detergent or iodophor (0.75% available iodine), injury levels between 50 and 90% were observed when

the organisms were grown on selective media, compared to growth on Tryptic Soy agar (TSA). Holding the LB samples at room temperature for one to two hours allowed resuscitation of the injured organisms for growth on the selective media. Holding the LB samples for greater than two hours resulted in the growth of the microorganisms.

The X_0 LB samples were plated immediately onto the agar media; the X_1 and X_2 LB samples were held 1.5 h at 20°C for recovery of any injured cells. Initial studies indicated that this treatment of the samples reduced the level of injury for growth on the selective media. X_0 samples were diluted 1:10 with 0.1% peptone water for pour plating with VRBA and overlayered with 5 ml of the VRBA medium. X_1 and X_2 samples were pour plated on VRBA without dilution. Prepoured plates of SPC, PAF and B-P were surface streaked with 0.1 ml aliquots of the samples. The two sets of VRBA plates were incubated separately at 35 and 45°C for 24 h. SPC and B-P plates were incubated at 35°C for 48 h, and PAF plates were incubated at 20°C for 72 h.

The data were calculated as ratios of the number of organisms released from the hands after washing, compared to the number released before washing. Mean counts and mean percentage change in number of bacteria released from hands were based on individual changes in count for each subject. The data were analyzed using $\log_{10}$ transformed ratios in a statistical computer package for Latin Square designs (BMDP2V, Biomedical Computer Programs, P-Series, 1979,

University of California Press).

C. Results

The microbiological techniques allowed five microbial parameters to be monitored, including transient and resident microflora. The SPC count was intended to determine the total transient and resident flora. The VRBA counts determined the efficacy of the agents against *E. coli* (incubated at 45°C) and total coliform-type bacteria (incubated at 35°C). The correlation between VRBA counts at 45 and 35°C was $r>0.98$, indicating that the transient *E. coli* strain inoculated onto the hands predominated the VRBA counts at both temperatures. Only the data for VRBA at 35°C are presented. PAF counts were used to indicate the efficacy of the agents against *P. fluorescens*. The *P. fluorescens* strain grew as distinctive fluorescent yellow colonies on the PAF medium. Only typical colony types on the PAF medium were included in the presumptive *P. fluorescens* count. Initially, typical colonies were isolated from the PAF plates and confirmed as *P. fluorescens* by comparison with the reference strains. The B-P medium was used to monitor *Micrococcaceae*-type organisms released from the hands, which were considered to represent typical resident microflora, as well as organisms possibly acquired from the ground beef. A summary of the probabilities of a significant effect attributable to agents is shown in Table 5.1.

Table 5.1. *Summary of probabilities (P) of a significant effect attributable to agents as a result of Latin Square design analyses of variance.*

Medium'	Experiment I.		Experiment II.	
	After 1x15-s wash	After 2x15-s washes	After 1x15-s wash	After 2x15-s washes
SPC	0.0415*	0.0468*	0.4218	0.4946
VRBA (35)	0.0239*	0.0290*	0.1612	0.3188
VRBA (45)	0.0450*	0.0061**	0.5113	0.5211
PAF	0.1743	0.3336	0.9876	0.0246*
B-P	0.3382	0.4651	0.2412	0.0534*

* Significant at the 95% confidence level ($P<0.05$)
** Significant at the 99% confidence level ($P<0.01$)

' Microbiological media: SPC = standard plate count agar; VRBA = violet red bile agar; PAF = Pseudomonas agar F; B-P = Baird-Parker medium.

The data for the percentage mean reduction in total count of transient and resident flora on the hands, monitored using SPC counts at 35°C, are shown in Table 5.2. The reduction in number of bacteria released from hands as a result of one and two successive 15-s washes was not impressive. All products gave reduced counts as a result of the treatments, implicating the transient flora in this measure. Only three agents (4% chlorhexidine gluconate, and the iodophor products containing 0.75 or 0.005% available iodine) achieved 80% or slightly greater reduction in count with one 15-s wash. Non-germicidal soap achieved a 75% reduction. After two successive 15-s washes, most agents (including the non-germicidal soap) achieved virtually 80% reduction in SPC counts. Only the 4% chlorhexidine gluconate and the Irgasan DP 300 (0.25%) washes gave better than 90% reduction. In Experiment I there was a significant effect attributable to agents (see Table 5.1).

Duncan's multiple range tests for differences among treatment means are shown in Table 5.3. On SPC medium, after one 15-s wash, only 4% chlorhexidine gluconate and iodophor (0.75% available iodine) showed a significantly greater decrease in count compared to the Irgasan DP 300 wash and the TCC bar soap. After two successive 15-s washes, only PCMX gave a significantly poorer result than the chlorhexidine and iodophor (0.75% available iodine) products. The rest of the agents, including the non-germicidal soap, were not significantly different from

Table 5.2. *Reduction in colony forming units (count on SPC medium) released from hands as a result of one or two successive 15-s hand wash treatments.*

Agent [1]	Initial count	After 1x15-s wash	Initial count	After 2x15-s washes
Experiment I.	mean count x10³ (percent) [2]			
A. Control soap	11.0	2.9 (75)	10.0	2.1 (79)
B. Chlorhexidine	11.0	1.8 (85)	9.4	0.3 (96)
C. Irgasan gel	7.6	2.7 (66)	8.5	1.6 (80)
D. Irgasan wash	9.9	4.3 (58)	9.4	1.0 (90)
E. PCMX	8.1	3.2 (65)	7.8	2.4 (74)
F. Iodophor	11.0	1.8 (81)	8.2	0.7 (88)
G. TCC	7.4	3.0 (56)	8.6	1.8 (80)
Experiment II.				
A'. Control soap	8.5	3.5 (48)	11.0	1.6 (82)
H. Iodophor wash	12.0	2.5 (80)	9.9	0.9 (84)
I. Iodophor dip	12.0	3.4 (66)	11.0	1.8 (84)
J. Hypochlorite dip	8.7	3.4 (62)	13.0	2.4 (81)
K. QAC dip	9.9	3.3 (67)	10.0	2.6 (76)

[1] Hand wash agents:

A and A' non-germicidal liquid hand soap;

B 4% chlorhexidine gluconate (Hibitane);

C and D Irgasan DP 300 (0.3%) gel and 0.25% hand wash;

E *Para*-chloro-*meta*-xylenol (PCMX) 0.325% hand wash;

F Iodophor (0.75% available iodine);

G 1% Trichlorocarbanilide (TCC) bar soap;

H Iodophor hand wash (0.005% available iodine);

I Iodophor hand dip containing 25 ppm available iodine;

J Sodium hypochlorite dip containing 50 ppm available chlorine;

K Quaternary ammonium (QAC) dip containing 930 ppm benzalkonium chloride.

[a] Mean counts and mean percentage change in number of bacteria released from hands are based on individual changes in count for each subject.

Table 5.3. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means on different microbiological media.* [1] [2] [3]

Experiment I

(i) SPC

After 1x15-s wash	F	B	A	C	E	D	G
After 2x15-s washes	B	F	D	A	C	G	E

(ii) VRBA

After 1x15-s wash	F	B	E	G	D	A	C
After 2x15-s washes	B	F	C	E	D	G	A

Experiment II

(i) PAF

After 2x15-s washes	H	A'	K	I	J

(ii) B-P

After 2x15-s washes	K	J	H	I	A'

[1] For key to product codes see Table 5.2.

[2] Microbiological media: SPC = standard plate count agar; VRBA = violet red bile agar; PAF = Pseudomonas agar F; B-P = Baird-Parker medium.

[3] Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

these two agents.

The results for the efficacy of the germicidal agents against *E. coli* are shown in Table 5.4. All agents, including the non-germicidal soap, gave greater than 90% reduction in count. Only 4% chlorhexidine gluconate achieved 99% reduction after two successive 15-s washes. A significant effect was attributed to agents in Experiment I (see Table 5.1). Duncan's multiple range tests for differences among treatment means (see Table 5.3) revealed that, after one 15-s wash, the iodophor (0.75% available iodine) gave a significantly greater reduction than all other agents, except 4% chlorhexidine gluconate. After the two 15-s wash sequences, significant differences were observed. However, 4% chlorhexidine gluconate was not significantly more effective against *E. coli* than the iodophor (0.75% available iodine), 0.3% Irgasan DP 300 gel and the PCMX (0.325%) antiseptic hand soap. These agents, except PCMX, gave a significantly greater reduction of *E. coli* compared to the non-germicidal soap.

The data for *P. fluorescens*, are shown in Table 5.5. Similar results to those for *E. coli* were observed, except that the TCC bar soap (one 15-s wash) and the dip methods of hand washing only gave 80 to 90% reduction in *P. fluorescens* count, whereas other agents gave greater than 90% reduction. The only significant effect attributable to agents in Experiment II was against *P. fluorescens* (see Table 5.1). Duncan's multiple range tests on these data indicated a

Table 5.4. *Efficacy of germicidal hand wash agents against* Escherichia coli *artificially inoculated onto hands from ground meat (based on count on VRBA medium incubated at 35°C).* [1]

Agent	Initial count	After 1x15-s wash	Initial count	After 2x15-s washes
Experiment I.	Mean count x10³ (percent)			
A. Control soap	8.4	0.6 (94)	6.0	0.4 (95)
B. Chlorhexidine	7.9	0.2 (97)	6.5	0.1 (99)
C. Irgasan gel	6.3	0.3 (92)	11.0	0.2 (98)
D. Irgasan wash	6.8	0.4 (93)	5.7	0.2 (97)
E. PCMX	5.0	0.2 (95)	3.4	0.1 (98)
F. Iodophor	6.3	0.1 (98)	3.6	0.1 (98)
G. TCC	6.1	0.3 (94)	7.8	0.2 (97)
Experiment II.				
A'. Control soap	7.2	0.3 (95)	8.8	0.2 (97)
H. Iodophor wash	9.7	0.4 (96)	5.8	0.1 (98)
I. Iodophor dip	11.0	0.5 (96)	8.4	0.2 (97)
J. Hypochlorite dip	5.7	0.4 (93)	10.0	0.4 (96)
K. QAC dip	8.9	0.3 (97)	8.2	0.4 (97)

[1] See footnotes to Table 5.2.

Table 5.5. *Efficacy of germicidal hand wash agents against* Pseudomonas fluorescens *artificially inoculated onto hands from ground meat (based on count on PAF medium).* [1]

Agent	Initial count	After 1x15-s wash	Initial count	After 2x15-s washes
Experiment I.	mean count x10² (percent)			
A. Control soap	4.3	0.2 (96)	3.4	0.1 (98)
B. Chlorhexidine	3.2	<0.1 (98)	2.5	<0.1 (98)
C. Irgasan gel	2.4	0.2 (93)	3.3	0.2 (95)
D. Irgasan wash	2.9	0.2 (95)	3.7	0.2 (93)
E. PCMX	2.9	0.1 (97)	2.6	<0.1 (99)
F. Iodophor	2.6	0.1 (96)	2.5	<0.1 (99)
G. TCC	2.7	0.4 (88)	3.2	0.2 (92)
Experiment II.				
A'. Control soap	3.5	0.2 (93)	3.6	0.2 (96)
H. Iodophor wash	4.1	0.2 (95)	3.1	0.1 (96)
I. Iodophor dip	3.9	0.3 (90)	3.8	0.5 (83)
J. Hypochlorite dip	3.9	0.6 (87)	4.2	0.8 (80)
K. QAC dip	3.5	0.5 (88)	4.4	0.5 (90)

[1]See footnotes to Table 5.2.

significantly greater decrease in *P. fluorescens* count attributable to the iodophor wash (0.005% available iodine) and non-germicidal soap compared to iodophor and hypochlorite dips (Table 5.3).

Changes in the "resident" microflora measured on B-P medium are shown in Table 5.6. These changes showed a marked difference to the trends for the "transient" microflora (Tables 5.2, 5.4 and 5.5). The non-germicidal soap caused a marked increase in the number of microorganisms released from hands after one or two successive 15-s washes. This applied also to most of the germicidal washes or dips except 4% chlorhexidine gluconate, iodophor (0.75% available iodine) and the QAC dip. The QAC dip treatment gave a significant reduction in number of microorganisms released from hands compared to iodophor dip and non-germicidal soap.

D. Discussion

The results of this study on transient bacteria inoculated onto hands from meats contrasts markedly with our earlier results for the total hand microflora (Sheena and Stiles, 1982, 1983a). However, the previous studies involved primarily the resident microflora. Both of the test organisms included in this study were gram negative bacteria, originally isolated from meat. *E. coli* was selected because of its role as an indicator organism and as a possible indication of what might happen to related pathogenic bacteria, such as *Salmonella* (Pether and Gilbert,

Table 5.6. *Mean count and percentage change in residual-type Micrococcaceae colony forming units released from hands after use of germicidal hand wash agents, measured by growth on Baird-Parker medium (based on count on B-P medium).* [1]

Agent	Initial count	After 1x15-s wash	Initial count	After 2x15-s washes
Experiment I.	mean count x10³ (percent)			
A. Control soap	0.9	1.2 (291)	0.7	0.4 (113)
B. Chlorhexidine	1.5	0.5 (83)	0.2	<0.1 (57)
C. Irgasan gel	0.6	0.4 (88)	0.2	0.3 (119)
D. Irgasan wash	0.4	0.6 (246)	0.1	0.3 (192)
E. PCMX	1.1	2.4 (275)	0.6	0.7 (160)
F. Iodophor	2.1	1.4 (83)	0.5	0.2 (74)
G. TCC	1.9	2.6 (134)	0.4	0.9 (158)
Experiment II.				
A'. Control soap	0.9	2.3 (313)	0.2	0.7 (263)
H. Iodophor wash	0.7	1.5 (226)	0.4	0.7 (155)
I. Iodophor dip	1.3	1.5 (137)	0.1	0.4 (242)
J. Hypochlorite dip	1.6	1.7 (246)	0.7	0.4 (234)
K. QAC dip	1.5	1.9 (84)	0.7	0.2 (68)

[1]See footnotes to Table 5.2.

1971). *P. fluorescens* was selected to indicate activity against spoilage-type bacteria. This particular strain was selected because of its pigmentation on PAF that made it easy to detect in mixed culture.

These transient bacteria inoculated onto hands from meats were markedly reduced by the short-exposure washes used in this study. The effective reduction or elimination of the transient microflora by non-germicidal soap has been widely reported (Ayliffe *et al.*, 1978; Lowbury *et al.*, 1964a; Ojajärvi, 1980; Sprunt *et al.*, 1973), however more recent studies with agents such as alcohol, povidone iodine and chlorhexidine have given better results than non-germicidal soaps (Ayliffe *et al.*, 1975; Dineen, 1978; Lilly and Lowbury, 1978; Ojajärvi, 1980). Our results confirmed the improved action of 4% chlorhexidine gluconate and iodophor (0.75% available iodine) against *E. coli* compared to the non-germicidal soap.

In our studies on resident microflora (Sheena and Stiles, 1982, 1983a), the efficacy of germicidal products containing Irgasan DP 300 or *para*-chloro-*meta*-xylenol (PCMX) for short-exposure hand washing was not satisfactory. However, against the transient bacteria in this study, these agents were far more effective. The trichlorocarbanilide (TCC) bar soap and the hand dips gave the least effective results against the transient bacteria. The results of this study, and our earlier study (Sheena and Stiles, 1982), favor hand washing above hand dipping techniques, both for

the bacteriological results as well as practical control.

The use of Baird-Parker (B-P) medium to monitor resident flora was justified by the similarity of these results compared to those obtained on the non-selective medium used in our previous study (Sheena and Stiles, 1982). Coagulase negative staphylococci are part of the resident microflora of skin (Price, 1938a; Seligmann and Rosenbluth, 1975). The preferential activity of QAC's against gram positive bacteria might account for the favorable result for the QAC dip against the "resident" flora. This confirmed our previous observation of the efficacy of a QAC dip (Sheena and Stiles, 1982).

Coliform bacteria and associated enteric pathogens are generally absent from skin, except in some special studies of food handlers (Pether and Gilbert, 1971). *Staphylococcus aureus* is generally associated with the nasal cavity (Williams, 1963), but it may be carried on skin as part of the transient microflora (Lowbury *et al.*, 1964a). The incidence of coagulase positive staphylococci is greater among meat handlers, with a tendency for these organisms to become part of the resident skin microflora (Roskey and Hamdy, 1972; Seligmann and Rosenbluth, 1975). This weighs in favor of the selection of germicidal hand washes as opposed to non-germicidal soaps for hygienic hand disinfection of food handlers. Bacteriologically, 4% chlorhexidine gluconate and iodophor (0.75% available iodine) remain the agents of choice for their better action against resident and

transient skin bacteria.

The 4% chlorhexidine gluconate product (Hibitane®) was selected as a reference agent for this study because of its use in medical practice. It is probably unsuitable for food handlers in this formulation. However, our studies with 4% chlorhexidine gluconate detergent solution confirmed its marked residual (substantive) effect (Sheena and Stiles, 1983a), and a need for special care in neutralizing its antibacterial activity for efficacy testing (Sheena and Stiles, 1982). Iodophor (0.75% available iodine) and equivalent products with relatively high concentrations of iodine are used in medical practice and by food handlers. There is resistance to the latter products because of color and odor, hence the low iodine products, such as product H (0.005% available iodine), warrant further study.

VI. COMPARISON OF BARRIER CREAMS AND GERMICIDES FOR HAND HYGIENE

This chapter is the text of a paper published by

A. Z. Sheena and M. E. Stiles,

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A. Introduction

Considerable emphasis is placed on hand hygiene in hospitals, nurseries and food handling establishments (Brodie, 1965; Ojajärvi, 1981; Seligmann and Rosenbluth, 1975; Steere and Mallison, 1975). Hand washing with ordinary soap (Lowbury *et al.*, 1964a; Sheena and Stiles, 1983b; Sprunt *et al.*, 1973) as well as hygienic hand disinfection (Ayliffe *et al.*, 1978; Ojajärvi, 1976; Sheena and Stiles, 1982) are recommended to reduce the number of microorganisms on hands and to prevent cross-contamination or infection (Berman and Knight, 1969). Germicidal hand wash agents containing 4% chlorhexidine gluconate or iodophor with 0.75% available iodine effectively reduce the number of bacteria released from hands (Ojajärvi, 1976; Sheena and Stiles, 1982, 1983a). Frequent use of germicidal hand wash agents has caused skin problems, including dry skin, irritation, chapping and dermatitis (Food and Drug Administration, 1974; Ojajärvi *et al.*, 1977). As a result, alternatives to these

agents have been sought, such as emulsion-type soaps (Ojajärvi, 1981) or sterile plastic gloves (Lowbury and Lilly, 1960; Price, 1938b). The latter are not considered satisfactory because the skin becomes occluded by the glove, leading to increased bacterial counts on the skin and increased chances of contamination from punctured or cracked gloves (Dyett, 1971; Lowbury and Lilly, 1960; Price, 1938b; Steel, 1980).

Barrier creams are widely used for skin protection (Green, 1974; Wedderburn, 1960). Hydrophobic barrier creams create a thin, water repellant layer over the skin (Wedderburn, 1960). This prevents aqueous liquids from contacting the skin, and may prevent microorganisms from being released from the skin. Some barrier creams are formulated with germicidal agents, for example benzalkonium chloride (Wedderburn, 1960), to reduce bacterial contamination of hands. Barrier creams are generally applied to clean, dry hands, following regular hand washing with ordinary soap (Anonymous, 1978; Green, 1974; Wedderburn, 1960). Although barrier creams are removed by hand washing their use is considered a useful adjunct to hand washing (Wedderburn, 1960).

The object of this study was to compare the ability of two commercial barrier creams to reduce the number of microorganisms released from hands with selected germicidal hand wash agents.

B. Materials and Methods

Two separate experiments using a replicated 4 x 4 and a single 7 x 7 Latin Square design were done. The sequence in which agents were used by subjects was randomly assigned by a specified procedure (Myers, 1972). Each subject was exposed to each agent over the period of the experiment, according to the designs shown in Table 6.1. The agents used in Experiment I included: (A) non-germicidal liquid hand soap; (B) iodophor containing 0.75% available iodine ("Tamed Iodine" Scrub®, West Chemicals Ltd., Montreal); (C) Protective Hand Cream #311® (West Chemicals Ltd., Montreal) (barrier I); and (D) "Debba" Wet Work Barrier Cream® (Deb Swarfega Ltd., Waterford, Ontario, Canada) (barrier II). Agent D was specially prepared by the manufacturer without the addition of 0.5% quaternary ammonium compound (QAC) as a bacterial inhibitor. The agents for Experiment II included agents A, B, C and D (above), (E) chlorhexidine gluconate (4%) liquid detergent (Hibitane®, Ayerst Laboratories Ltd., Montreal); (F) iodophor hand wash containing 0.005% available iodine; and (G) an antibacterial skin cleanser containing 0.3% Irgasan DP 300 in gel.

Washing procedures in both experiments were identical and followed the methods previously described by Sheena and Stiles (1982) using 15-s exposure time. Barrier cream was applied as an adjunct treatment to non-germicidal hand washing. Hands were washed for 15 s with non-germicidal soap, dried, and approximately 0.3 g of the barrier cream

Table 6.1. *Latin Square designs used for this study.* [1]

(i) Experiment I (replicated 4 x 4 design)

Day	I	II	III	IV
Subjects	Agents			
11 and 21	B	D	C	A
12 and 22	A	C	B	D
13 and 23	D	B	A	C
14 and 24	C	A	D	B

(ii) Experiment II (7 x 7 design)

Day	I	II	III	IV	V	VI	VII
Subject	Agents						
1	C	D	B	F	E	G	A
2	D	B	A	G	C	E	F
3	E	C	D	A	B	F	G
4	G	F	C	B	D	A	E
5	F	G	E	D	A	B	C
6	B	A	G	E	F	C	D
7	A	E	F	C	G	D	B

[1]Hand wash agents and barrier creams:

A Non-germicidal liquid hand soap;

B Iodophor (0.75% available iodine);

C Protective Hand Cream #311 (barrier I);

D "Debba" Wet Work Barrier Cream (barrier II);

E Chlorhexidine gluconate (4%) liquid detergent (Hibitane);

F Iodophor hand wash (0.005% available iodine);

G 0.3% Irgasan DP 300 in gel.

was applied to the clenched finger tips of each hand. In a procedure resembling the 15-s hand wash technique, the cream was spread over the hands, and allowed to dry for an additional 15 s.

Sampling was done by finger imprint technique onto separate Letheen agar (LA; Difco) plates for each hand (Sheena and Stiles, 1982, 1983a). Samples were taken before treatment (Y_0), and after treatment (Y_1). The inocula were spread using a sterile, glass "hockey stick", and the plates were incubated at 35°C for 24 h. The mean number of microorganisms released from the finger tips was calculated from the plate counts for the left and right hands after each treatment.

The persistence of the effect of barrier creams was measured by finger tip sampling after two additional treatments: (i) after a 15-s rinse under running tap water (Y_2); and (ii) after a 15-s wash with non-germicidal soap (Y_3). Based on the results of these experiments, an additional study was done to determine the persistence of barrier cream and germicide treatment effects. Hands were exposed to agents A to E above, and subjected to 12 consecutive 15-s rinses under running tap water. Finger tip imprints on LA were done after each rinse. The plates were handled as described above and changes in the counts were determined relative to the initial number of microorganisms released from the finger tips. A total of ten subjects was involved in this experiment. Each subject used each agent on

one occasion during the experiment.

Data were calculated as the ratio of the number of microorganisms released from finger tips after treatment compared to the number released before treatment. Mean counts and the mean percentage of microorganisms released from finger tips were based on the individual changes in count for each subject. Data were analyzed using $\log_{10}$ transformed ratios in a statistical computer package for Latin Square designs (BMDP2V, Biomedical Computer Programs, P-series, 1979, University of California Press).

C. Results

The mean number of microorganisms released from finger tips and the percentage released after treatment with the barrier creams or germicidal hand wash agents are shown in Table 6.2. In both experiments a significant effect ($P<0.001$) was attributed to treatments. The barrier creams, iodophor (0.75% available iodine) and 4% chlorhexidine gluconate treatments resulted in a significant decrease in number of microorganisms released. This is illustrated by the results for Duncan's multiple range test shown in Table 6.3. The other agents, including non-germicidal soap, iodophor hand wash containing 0.005% available iodine and the Irgasan DP 300 gel resulted in an increase in the number of microorganisms released from the finger tips.

Persistence of the barrier creams measured by release of microorganisms from the finger tips after a water rinse

Table 6.2. *Mean change in colony forming units released from finger tips after 15-s hand washing or barrier cream application.* [1]

Agent	Initial	After treatment
	Number of microorganisms x10 (percent) [2]	
Experiment I		
A Control soap	14.6	21.4(155)
B Iodophor	12.2	4.8(42)
C Barrier cream I	7.9	1.7(41)
D Barrier cream II	6.4	3.7(59)
Experiment II		
A Control soap	8.9	13.1(148)
B Iodophor	11.9	6.5(54)
C Barrier cream I	9.2	4.5(48)
D Barrier cream II	9.8	6.0(56)
E Chlorhexidine	7.7	3.3(42)
F Iodophor wash	6.8	7.5(116)
G Irgasan gel	8.7	10.5(126)

[1] Explanation of product codes given in Table 6.1.

[2] Mean counts and mean percentage survivors calculated from individual changes in count for each subject after 15-s hand washing or barrier cream application (Y_1) compared to first sampling, before treatment (Y_0).

Table 6.3. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means for 15-s hand washing or barrier cream application.* [1] [2]

Experiment I:	C	B	D	A			
Experiment II:	E	C	B	D	F	G	A

[1] Explanation of product codes given in Table 6.1.

[2] Agents underlined with an unbroken line are not statistically different at 95% confidence level.

and a subsequent wash with non-germicidal soap are shown in Table 6.4. The analysis of variance indicated a significant effect ($P<0.01$) attributable to treatments (water rinse and soap wash). The effect of the barrier creams was diminished after rinsing with water, and after washing with soap, the number of microorganisms released was markedly increased. This was confirmed by the results for Duncan's multiple range test shown in Table 6.5. A significant increase in the number of microorganisms released from hands occurred as a result of the water rinse and soap wash.

Persistence of the barrier creams and effective germicides was studied by determining the change in number of microorganisms released from finger tips after each of the twelve consecutive water rinses. The mean percentages of the number of microorganisms released from the finger tips of the ten subjects are plotted in Figures 1 and 2. The non-germicidal soap caused an increase in number of microorganisms released from finger tips. Subsequent water rinses gave counts greater than the initial number released (Y_o). In contrast, a marked reduction in count was observed after adjunct treatment with the barrier creams. The reduction of this effect was confirmed by the increase in number of microorganisms released after rinsing with water. This was more marked with barrier II than with barrier I. However, after six rinses the number of microorganisms released from finger tips using either barrier cream was equivalent.

Table 6.4. *Persistence of barrier cream effect tested by water rinse and soap washing after treatment.* [1]

Agent	Initial number released (x10)	Percent of microorganisms released After treatment	Water rinse	Soap wash
Experiment I				
Barrier cream I	7.9	41	67	86
Barrier cream II	6.4	59	86	106
Experiment II				
Barrier cream I	9.2	48	73	92
Barrier cream II	9.8	56	83	96

[1] See footnotes for Table 6.2

Table 6.5. *Summary of Duncan's multiple range test (95% confidence level) for persistence of treatment effects.* [1] [2]

Experiment I:	Y_1	Y_2	Y_3
Experiment II:	Y_1	Y_2	Y_3

[1] Explanation of codes: Y_1 = application of barrier cream; Y_2 = hand rinse with tap water for 15-s; Y_3 = hand wash with non-germicidal soap for 15-s.

[2] Treatments underlined with an unbroken line are not statistically different at 95% confidence level.

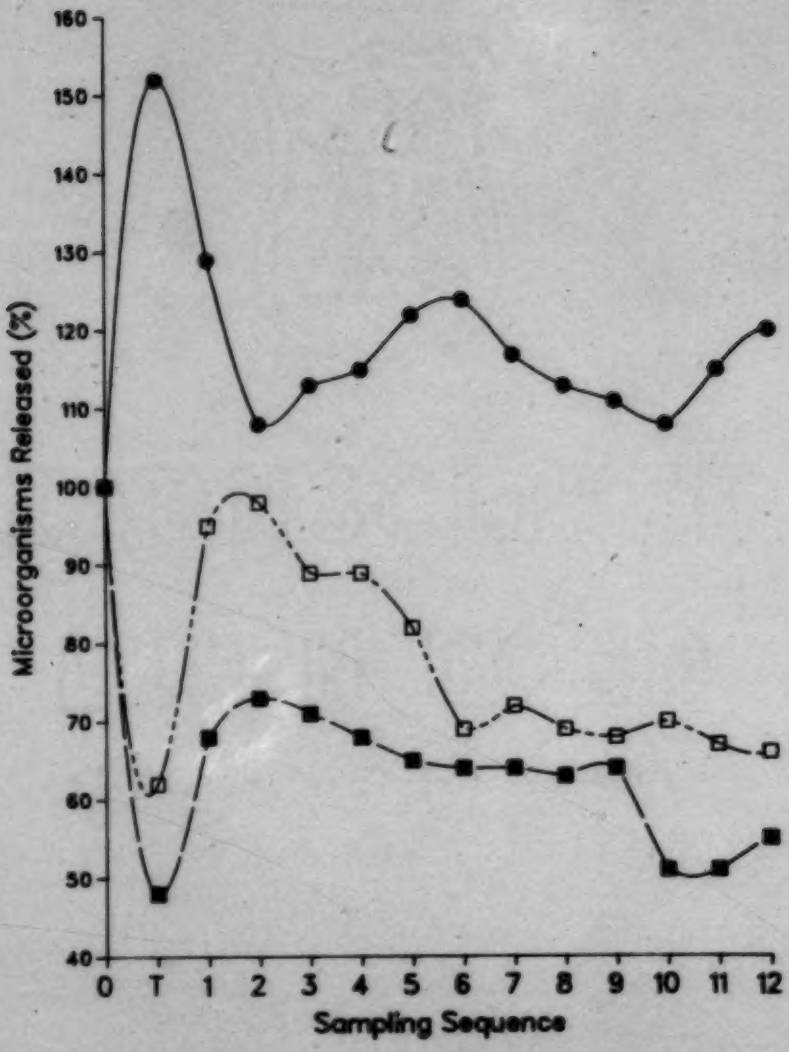

Figure 6.1. Microorganisms released (percent) from finger tips as a result of initial non-germicidal hand wash and barrier cream application, followed by successive water rinses.

• = non-germicidal liquid hand soap;
▪ = Protective Hand Cream #311 (barrier I);
▫ = "Debba" Wet Work Barrier Cream (barrier II)
0 = initial sample in sampling sequence,
T = sample taken after treatment, before the sequence of water rinses.

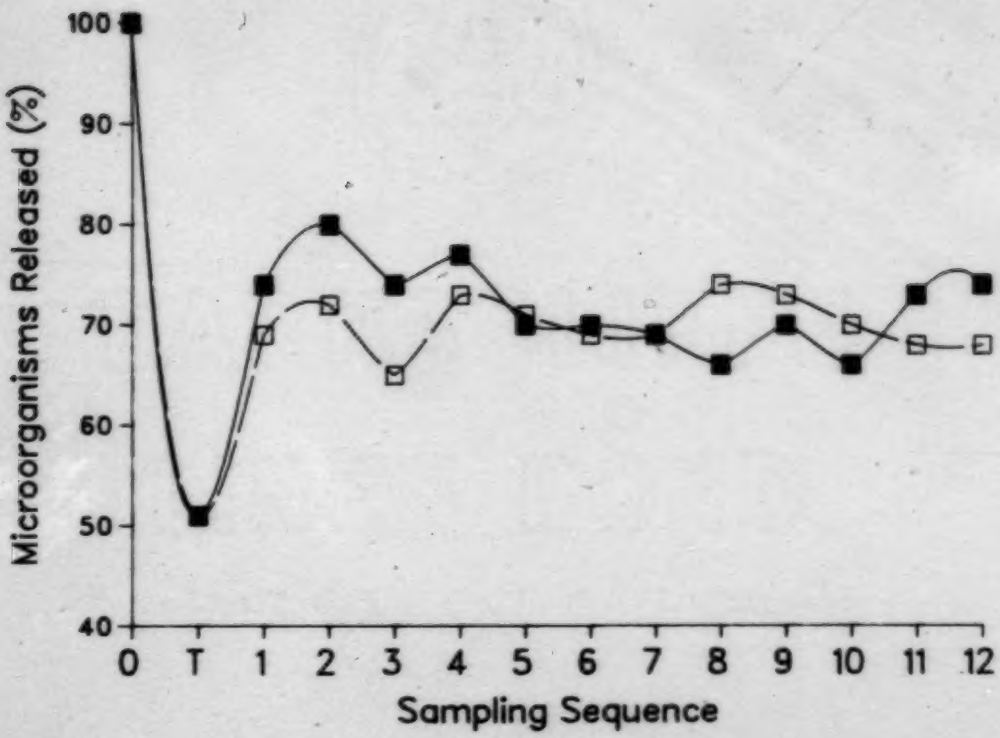

Figure 6.2. Microorganisms released (percent) from finger tips after treatment with germicidal hand wash agents, followed by successive water rinses.
■ = Iodophor (0.75% available iodine);
▫ = Chlorhexidine gluconate (4%) liquid detergent (Hibitane).
0 = initial sample in sampling sequence,
T = sample taken after treatment, before the sequence of water rinses.

This apparent loss of the "barrier" effect prompted a similar study of the persistence effect of iodophor (0.75% available iodine) and 4% chlorhexidine gluconate during successive rinses (Figure 2). The marked decrease in microorganisms released is apparent, however, with both germicides a subsequent increase in counts was observed. This was equivalent to the increase in count observed for barrier cream I.

D. Discussion

Previous studies indicated only a limited number of germicidal hand wash agents that were likely to reduce the number of microorganisms released from hands after short exposure (15-s) hand washes (Sheena and Stiles, 1982, 1983a). The barrier creams used in this study were designed to protect hands from direct contact with moisture. It was assumed that they might also prevent or reduce the release of microorganisms from hands. Since these products are intended to protect hands from dryness, chapping and dermatitis (Green, 1974), if the release of microorganisms is reduced, a dual protective effect of skin and foods might be achieved.

The barrier creams reduced the number of microorganisms released from finger tips, equivalent to the most effective germicide products (iodophor with 0.75% available iodine or 4% chlorhexidine gluconate), reported in our previous studies (Sheena and Stiles, 1982, 1983a,b). Further

evaluation of the efficacy of the barrier creams depended on their continued prevention of microorganism release from finger tips. Water rinsing was selected as the treatment to challenge the persistence of the "barrier". This was predicated on successive water rinses simulating handling of foods.

The water rinses revealed an unexplained difference between the barrier creams, suggesting that differences might occur as a result of product formulation. However, the two barrier creams gave an equivalent protective effect after the sixth successive water rinse, and their effect was similar to that of the effective germicidal agents.

Wedderburn (1960) used barrier cream as an adjunct to hand hygiene, and noted that a valuable antiseptic effect could be achieved with 0.5% benzalkonium chloride in the cream. In our study, barrier creams were selected that specifically excluded antiseptic agents such as QACs. The marked reduction in microorganisms released from finger tips suggests the possibility that effective hand hygiene and skin protection might be achieved for food handlers with specially prepared barrier creams. Transient bacteria were not specifically included in this study, however our earlier report (Sheena and Stiles, 1983b) indicated that transient bacteria on hands are more readily reduced than resident bacteria.

VII. LOW CONCENTRATION IODOPHORS FOR HAND HYGIENE

This chapter is the text of a paper to be submitted for publication in the Journal of Hygiene, Cambridge, by A. Z. Sheena and M. E. Stiles.

A. Introduction

Iodophors have been recommended for use as antibacterial agents for many purposes, including hand disinfection (Shelanski and Shelanski, 1956). Davis (1971) in a review of the use of iodophors in food and beverage manufacture, reported that iodophors are suitable for hand hygiene. Several studies on the germicidal efficacy of iodophors have evaluated the efficiency of high concentration (0.75% available iodine) products (Davies *et al.*, 1977; Joress, 1962; Ojajärvi, 1976; Peterson *et al.*, 1978; Van der Hoeven and Hinton, 1968). Earlier studies by Sheena and Stiles (1982,1983a) indicated that the only effective hand wash agents for the reduction of the resident skin microflora released from hands after washing were an iodophor hand wash containing 0.75% available iodine and 4% chlorhexidine gluconate liquid detergent. Similarly, the transient microflora (*E. coli*) inoculated onto hands in ground meat was more effectively reduced by these two agents than by other agents tested (Sheena and Stiles, 1983b).

Although the 4% chlorhexidine gluconate product might be acceptable for use in food handling, its principal orientation is for hospital use. The iodophor products were also developed for hospital use, but they have been more broadly adapted for various agricultural and food handling applications.

Iodophor products containing 0.75% iodine create some user resistance, because of odor and color of the product when it is applied to the skin. As a result, a new generation of low available iodine iodophor products have been developed (Berkelman *et al.*, 1982; U. S. Patent, 1981). One of these products was included in our earlier studies (Sheena and Stiles, 1983a,b,c), but the results achieved were equivocal. The iodophor product containing 0.005% available iodine was not significantly different from the 0.75% iodophor product under some conditions, but this could not be generally concluded (Sheena and Stiles, 1983a). In seeking a germicidal hand wash agent for food handlers, the testing criteria were: that the agent should reduce the number of microorganisms released from hands; that transient microorganisms inoculated onto hands should be more effectively reduced by the agent than by non-germicidal soap; and that products should be readily accepted for use by food handlers.

The objective of this study was to determine the efficacy of a range of iodophor products, containing 0.75, 0.5, 0.3, 0.1 and 0.005% available iodine compared to a

non-germicidal soap and chlorhexidine gluconate liquid detergents containing 4 or 2% active ingredient.

B. Materials and Methods

The methods used in this study were similar to those described in an earlier study by Sheena and Stiles (1983b). A repeated 9 x 9 Latin Square design was done, using different subjects in each of the experiments. The sequence in which agents were used by each subject was randomly assigned by a specified procedure (Myers, 1972), and each subject was exposed to all of the agents over the course of the experiment. Subjects used the assigned agent for one 15-s exposure using a standardized hand washing procedure (Sheena and Stiles, 1982). There were three testing days per week (Monday, Wednesday and Friday) so that three products were tested on each subject, each week.

The nine agents were: (A) non-germicidal liquid hand soap; (B) and (C) chlorhexidine gluconate 4% and 2% liquid detergents, respectively (Ayerst Laboratories, Montreal, Canada); (D) to (H) iodophor hand wash agents containing 0.75, 0.5, 0.3, 0.1 and 0.005% available iodine, respectively, (West Chemicals Ltd., Montreal, Canada); and (I) tap water.

Hands were contaminated with *E. coli* and *Pseudomonas fluorescens* that had been inoculated into ground beef to give 10^4 and 10^7 c.f.u./g, respectively, for the first experiment; and 10^7 and 10^8 c.f.u./g, respectively, for the

second experiment. Counts were increased in the second experiment to give an increased level of contamination of the test organisms on hands. The ground beef was checked each day to determine its microbiological quality, including total aerobic plate count, coliform, *E. coli*, *P. fluorescens* and total "gram positive cocci" counts. The levels of *E. coli* and *P. fluorescens* inocula in the ground beef were also determined. The inoculated ground beef was dispensed in two 50g amounts in separate sterile petri dishes for use as the inoculum for the finger tips.

Subjects rinsed their hands with 5 ml of 95% ethanol containing 1% glycerol, and hands were rubbed together until dry. Finger and thumb tips were pushed into, and held in the ground beef inoculum for 5 s, and the inoculum was distributed over the hands by rubbing up to the wrists, until the hands were dry. One of the hands was randomly selected for sampling for the initial count (X_0), by rinsing in 100 ml letheen broth (LB, Difco) in a plastic bag (28.5 x 12.5 x 7.5 cm, 25 mil, Polyrama Plastics Ltd., Edmonton, Canada) using the standard hand rinse method (Sheena and Stiles, 1982). Hand washing was also done according to standardized procedures that were previously described (Sheena and Stiles, 1982). After the initial sample (X_0) had been taken, the hand was rinsed under flowing tap water to remove residues of LB. The sample after the 15-s wash (X_1) was taken from the alternate hand by rinsing in LB. Subjects rinsed their hands with the glycerol in ethanol solution

after the sampling period had been completed.

Bacteriological testing of the hand rinse samples was done by plating in duplicate onto the following Difco media: violet red bile agar (VRBA), Pseudomonas agar F (PAF) and Baird-Parker medium (B-P). The X_0 LB samples were plated without delay onto the agar media; however, the X_1 LB samples were plated after they had been held at 20°C for 1.5 h, for resuscitation of injured cells. VRBA plates were incubated at 45°C for 24 h, B-P plates at 35°C for 48 h, and PAF plates at 20°C for 72 h.

The data were calculated as ratios of the number of microorganisms released from hands after washing, compared to the number released before washing. Mean counts and percentage mean reduction or change in number of microorganisms released from hands were based on individual changes in count for each subject. The data were analyzed using $\log_{10}$ transformed ratios in a statistical computer package for Latin Square designs (BMDP2V, Biomedical Computer Programs, P-Series, 1979, University of California Press).

C. Results

The available iodine content of the iodophor germicides was confirmed by titration. Most agents contained a slight excess of available iodine, compared to the manufacturer's listed concentration, however the 0.005% product contained 0.01% available iodine (see Table 7.1).

Table 7.1. *Available iodine concentration of iodophor germicides for hand hygiene.*

Manufacturer's label (%)	Titratable iodine (%)	Percent difference'
0.75	0.78	4.0
0.5	0.56	12.0
0.3	0.32	6.7
0.1	0.13	30.0
0.005	0.01	100.0

'Percentage difference bewtween titratable iodine and manufacturer's indicated iodine concentration.

The microbiological techniques allowed three microbial parameters to be measured, including *E. coli* on VRBA, *P. fluorescens* on PAF and Micrococcaceae-type 'resident' bacteria on B-P medium.

The reduction of *E. coli* on hands as a result of exposure to the hand wash agents is shown in Table 7.2. All agents, including non-germicidal soap, gave a mean reduction in *E. coli* of greater than 95%. The tap water rinse, however, resulted in less than 80% reduction in *E. coli*. Analyses of variance indicated a significant effect ($P<0.001$) attributable to agents. The Duncan's multiple range test (95% confidence levels) for differences among treatment means are shown in Table 7.3. Slight differences were observed between the two experiments. In both cases, however, all agents gave a significantly better reduction in *E. coli* than the tap water rinse. The greatest decrease in *E. coli* was observed with 4% chlorhexidine gluconate liquid detergent and iodophor containing 0.75% available iodine. In experiment I, these products were significantly better than non-germicidal soap and iodophor containing 0.1% available iodine. In experiment II, the chlorhexidine (4%) and iodophor (0.75%) were significantly better than all other agents.

The equivalent data for *P. fluorescens* are shown in Tables 7.4 and 7.5. Washing hands with any of the agents, including the non-germicidal soap, resulted in greater than 90% reduction in *P. fluorescens*. Only the tap water rinse

Table 7.2. *Efficacy of germicidal hand wash agents against* Escherichia coli *artificially inoculated onto hands from ground meat (based on count on VRBA medium).*[1]

Agent	Experiment I			Experiment II		
	Initial count	Count after 15-s wash		Initial count	Count after 15-s wash	
	mean count x10³		(%)	mean count x10³		(%)
A. Control Soap	4.8	0.2	(95.5)	5.0	0.2	(96.3)
B. Chlorhexidine (4%)	5.5	0.1	(98.0)	6.0	0.08	(98.9)
C. Chlorhexidine (2%)	5.0	0.2	(97.4)	6.0	0.2	(97.0)
D. Iodophor (0.75%)	4.6	0.1	(97.9)	6.6	0.05	(99.2)
E. Iodophor (0.5%)	3.9	0.2	(96.4)	5.1	0.1	(97.9)
F. Iodophor (0.3%)	5.0	0.2	(96.3)	6.3	0.2	(97.5)
G. Iodophor (0.1%)	5.0	0.3	(95.2)	6.2	0.1	(97.9)
H. Iodophor (0.01%)	5.0	0.2	(96.3)	6.3	0.2	(97.3)
I. Tap water	4.1	1.1	(72.1)	4.6	1.0	(78.4)

[1] Mean counts and mean percentage reduction in number of bacteria inoculated onto hands are based on individual changes in count for each subject.

Table 7.3. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means (based on count on VRBA medium).*[1] [2]

Experiment I:

B D C E F H A G I

Experiment II:

D B E G F H C A I

[1]For key to product codes see Table 7.2.

[2]Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

Table 7.4. *Efficacy of germicidal hand wash agents against* Pseudomonas fluorescens *artificially inoculated onto hands from ground meat (based on count on PAF medium).*[1]

Agent	Experiment I			Experiment II		
	Initial count	Count after 15-s wash		Initial count	Count after 15-s wash	
	mean count x10^2		(%)	mean count x10^3		(%)
A. Control soap	5.0	0.4	(92.8)	3.0	0.1	(96.5)
B. Chlorhexidine (4%)	6.2	0.1	(98.8)	4.0	0.04	(99.1)
C. Chlorhexidine (2%)	4.7	0.2	(95.6)	3.6	0.1	(97.1)
D. Iodophor (0.75%)	5.7	0.1	(98.8)	3.4	0.02	(99.5)
E. Iodophor (0.5%)	4.9	0.4	(95.4)	3.2	0.06	(97.8)
F. Iodophor (0.3%)	6.2	0.6	(96.1)	3.5	0.1	(97.4)
G. Iodophor (0.1%)	5.2	0.3	(94.1)	3.3	0.1	(97.2)
H. Iodophor (0.01%)	5.4	0.4	(93.2)	4.1	0.07	(97.8)
I. Tap water	4.1	1.5	(63.1)	3.0	0.9	(71.3)

[1] See footnotes to Table 7.2.

Table 7.5. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means (based on count on PAF medium).*[1] [2]

Experiment I:

B D F C E G H A I

Experiment II:

D B E H F G C A I

[1]For key to product codes see Table 7.2.

[2]Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

failed to achieve a 90% reduction in *P. fluorescens*. Analyses of variance indicated a significant effect ($P<0.001$) attributable to agents. This was contributed in part by the difference between the tap water rinse and hand wash treatments, however 4% chlorhexidine gluconate liquid detergent and iodophor containing 0.75% available iodine were generally significantly better than all other agents. In experiment II, 4% chlorhexidine gluconate was not significantly better than the iodophor agents containing 0.5 and 0.01% available iodine.

The change in 'resident' microorganisms released from hands as a result of the 15-s wash are shown in Table 7.6. The mean percentage change in number of microorganisms released from hands increased with the non-germicidal soap, the tap water rinse and iodophor products containing 0.1 and 0.01%. For some agents there were marked differences between subjects, for example with 2% chlorhexidine gluconate, 7 subjects had decreased counts while 10 subjects had increased counts and one had no change as a result of washing. In contrast, with 4% chlorhexidine gluconate 15 out of the 18 subjects had decreased counts of microorganisms released from hands after washing (2 subjects showed no change in count), with iodophor containing 0.75% available iodine 10 out of 18 subjects had decreased counts (4 subjects showed no change in count), while washing with the non-germicidal soap resulted in 14 out of 18 subjects had increased counts (3 subjects showed no change in count).

Table 7.6. *Mean count and percentage change in residual-type* Micrococcaceae *colony forming units released from hands after use of germicidal hand wash agents, measured by growth on Baird-Parker medium (based on count on B-P medium).*[1]

Agent	Experiment I			Experiment II		
	Initial count	Count after 15-s wash		Initial count	Count after 15-s wash	
	mean count x10²		(%)	mean count x10²		(%)
A. Control Soap	2.0	2.6	(166)	1.8	3.2	(167)
B. Chlorhexidine (4%)	1.5	0.8	(46.4)	2.6	1.4	(46.8)
C. Chlorhexidine (2%)	2.7	1.9	(69.5)	2.0	1.8	(95.8)
D. Iodophor (0.75%)	1.2	1.0	(73.5)	1.3	0.8	(68.5)
E. Iodophor (0.5%)	2.1	1.5	(72.4)	0.9	0.6	(68.8)
F. Iodophor (0.3%)	2.3	1.5	(60.8)	2.1	1.2	(57.2)
G. Iodophor (0.1%)	1.4	2.1	(148)	1.2	1.5	(130)
H. Iodophor (0.01%)	2.0	2.2	(109)	1.7	1.4	(77.0)
I. Tap water	0.8	1.8	(227)	1.2	2.8	(240)

[1] Mean counts and mean percentage change in number of bacteria released from hands are based on individual changes in counts for each subject.

Analyses of variance indicated a significant effect attributable to agents ($P<0.01$). The results of the Duncan's multiple range test are shown in Table 7.7. Differences between agents were less distinct for the resident microflora than for the transient microflora (see Tables 7.3 and 7.5). The only differences that can be interpreted for these data are that the 4% chlorhexidine gluconate wash significantly reduced the number of resident microorganisms released, compared to non-germicidal soap, iodophor containing 0.1% available iodine and the tap water rinse. Iodophor containing 0.3% available iodine significantly reduced the number of microorganisms released from hands compared to non-germicidal soap and the tap water rinse. However, there was no significant difference between the iodophor products.

D. Discussion

Many studies on the efficacy of germicidal agents for hand hygiene have used the resident microflora of the skin as the basis for evaluation. Ojajärvi (1980) emphasized the importance of the transient microflora in such evaluations, because of their significance to the transmission of pathogens. In this study, an attempt has been made to consider both the resident and transient microorganisms. Potentially pathogenic or indicator microorganisms are represented by *E. coli* and potential spoilage microorganisms by *P. fluorescens*. Although resident microorganisms might

Table 7.7. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means (based on count on B-P medium).*[1] [2]

Experiment I:

B F C E D H G A I

Experiment II:

B F D E H C G A I

[1] For key to product codes see Table 7.2.

[2] Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

include *Staphylococcus aureus*, most resident skin microorganisms detected by aerobic culturing techniques are the non-pathogenic, Micrococcaceae-type bacteria that would be expected to grow on Baird-Parker medium for *S. aureus* (Noble and Somerville, 1974; Sheena and Stiles, 1982; Stiles and Ng, 1981). As a result, *E. coli* and *P. fluorescens* were inoculated into meat to contaminate hands, and samples were cultured not only onto selective media for *E. coli* and *P. fluorescens*, but also onto Baird-Parker medium.

A washing time of 15 s was used in this study, because it was considered more likely to represent hand hygiene practice in food handling than the longer 30 s washing time used in many hand hygiene studies involving hospital workers (Ayliffe *et al.*, 1978; Lilly and Lowbury, 1978; Lowbury *et al.*, 1964a; Ojajärvi, 1976). Ojajärvi (1980) also used a 15-s wash in a study of hand hygiene of nurses, because 15 s was observed to be more representative of hand washing times in ward practice. A water rinse was included in our study because many food handlers were observed to forego a soap wash in favour of a brief water rinse.

The study was designed as a repeated 9 x 9 Latin Square, using different subjects for each experiment. The only other change in the second experiment was a ten-fold increase in the number of *E. coli* and *P. fluorescens* used to inoculate the ground meat, and hence the hands. This resulted in an increased sensitivity of the test, from 95-99% to 99.5-99.9%. The repeated experiment served as a

useful confirmation of the data. The reductions of *E. coli* and *P. fluorescens* and the changes in number of 'resident' microorganisms released from hands after washing were consistent with earlier observations (Sheena and Stiles, 1983b).

Chlorhexidine gluconate (4%) liquid detergent (Hibitane) and an iodophor hand wash containing 0.75% available iodine (Prepodyne or "Tamed Iodine Scrub") had been shown to be effective against both of these transient bacteria (Sheena and Stiles, 1983b), and they also reduced the number of (resident) bacteria released from hands after short exposure hand washing (Sheena and Stiles, 1982). They were the only agents tested that met the criterion of reduction of both transient and resident microflora released from hands after washing. The iodophor product containing 0.75% available iodine arouses some user resistance, because of its strong odour, colour and short term staining effect. As a result, a new generation of iodophor products, with lower available iodine contents have been developed (West Design Chemical Group, West Agro-Chemical Inc., Westwood, Kansas, U.S.A.). These products are intended to overcome user resistance to iodophors.

Earlier studies, incorporating an iodophor with 0.005% available iodine, gave equivocal results (Sheena and Stiles, 1983 a,b). Dilute preparations of iodophor solutions were shown to be more effective against certain microorganisms than high concentration (10%) solutions in *in vitro* studies

(Berkelman *et al.*, 1982). New iodine germicidal compostions with low available iodine contents down to 0.01% have been patented (U.S. Patent, 1981) for various purposes including germicidal hand wash agents. A range of iodophor products with available iodine concentrations ranging between 0.75 and 0.01% were included in this study to determine their *in vivo* efficacy, according to the system used in our earlier studies. A 2% chlorhexidine gluconate liquid detergent, which is used in general hospital hygiene, was also incorporated into the study as a possible alternative to 4% chlorhexidine gluconate or the iodophors.

All agents markedly reduced the transient bacteria inoculated onto the hands. Only 4% chlorhexidine gluconate and iodophor containing 0.75% available iodine gave improved reduction of *E. coli* and *P. fluorescens* compared to the other agents. With all other agents significantly better than tap water, but not significantly different from non-germicidal soap, the value of intermediate and low concentration iodophor products, as well as the 2% chlorhexidine gluconate product, might well be questioned compared to use of a non-germicidal soap. The differentiation of the products could rely on control of the resident microflora.

Unfortunately, the counts on Baird-Parker medium as a measure of resident microflora were not clear. It appeared that the non-germicidal soap and the lower concentration iodophor agents were equivalent to the water rinse, actually

increasing the number of microorganisms released from hands after a 15-s wash. However, many other agents, including iodophor agents and 2% chlorhexidine gluconate were not significantly different from 4% chlorhexidine gluconate in their effect on the 'resident' microflora. The Baird-Parker medium counts, representing the resident-type microflora, had a high variance, making the distinction between products difficult. This differed from earlier results (Sheena and Stiles, 1982), in which the resident microflora was tested by plating onto a non-selective growth medium. The high variability of the data for resident-type microflora on Baird-Parker medium and use of a non-selective medium requires resolution. The results of this study indicate that the best choice of hand wash agent would be 4% chlorhexidine gluconate liquid detergent or iodophor containing 0.75% available iodine.

VIII. CONCLUSIONS

Hand washing is an integral part of sanitation programmes for food handlers. It is necessary, not only to reduce the possibility of contaminating foods with potential pathogens and spoilage microorganisms, but also for aesthetic reasons. It is often assumed that any hand germicide will achieve the required control of microorganisms on hands. Indeed, this expectation may be heightened by the requirement for germicidal hand wash agents in Canada to carry a Drug Identification Number (D.I.N.) issued by National Health and Welfare. This registration relies more on *in vitro* test results than *in vivo* efficacy test results. D.I.N. registration, therefore, is not an assurance of an efficacious product.

The problem is further influenced by the fact that hand washing for food handlers differs markedly from the rigid requirements for surgeons in preoperative scrubs or for nurses in control of cross-infection of high risk patients. Nonetheless, nursing practice can be more closely identified with food handling requirements than surgical practice. Many studies of nurses have involved relatively long wash times, from 30 seconds to 2 minutes duration. Such times would be considered impractical for food handlers, and Ojajärvi (1980) reduced the exposure time in a nursing study to 15 s, "because the average washing time in wards is closer to that

time".

The best method of sampling hands for bacteriological analysis remains unresolved. The finger tip imprint technique and the hand rinse method used in these studies are known to represent a small percentage of bacteria on the skin. A study by Selwyn and Ellis (1972) indicated that 0.2% of bacteria were released by a direct agar contact technique. However, the criterion for judging efficacy of germicidal agents was not the reduction of the bacteria on the hands, but the reduction of the number of bacteria released from hands after washing. The sampling technique (finger tip imprint or hand rinse) used in these studies was selected according to the design and needs of the experiment. In the first experiment, both sampling techniques were used. The bacterial counts from the two methods were not highly correlated, however agents that were considered good by the one sampling method were similarly good by the other sampling method. Over the series of experiments, the methods used were judged to be successful because of their reproducibility.

In studies of the resident microflora on hands, letheen agar (Difco) was used to sample and grow the bacteria by the finger imprint technique, and letheen broth (Difco) was used as the rinse in the hand rinse technique, and dilutions were plated onto standard plate count agar (Difco). The use of letheen agar or broth was shown to neutralize the active compounds in the hand wash agents. The bacteria able to grow

under these conditions would be the aerotolerant mesophiles. The majority of the bacteria growing on the plates after the washing techniques were the *Micrococcaceae*-type bacteria, associated with the resident aerobic microflora of the skin.

Studies of transient bacteria inoculated onto hands involved the use of relatively large numbers of bacteria and selective media for the detection of these bacteria. As a result, non-selective media could not be used to determine the changes in resident microflora released from hands at the same time that transient bacteria were being monitored. The use of Baird-Parker to monitor the *Micrococcaceae*-type resident microflora gave similar results to earlier studies, but the variation in the data between subjects resulted in the data having a higher variance than previously observed. The value of Baird-Parker medium to monitor the resident-type microflora requires further study.

Throughout the studies, the most effective agents against resident and transient microorganisms were 4% chlorhexidine gluconate and iodophor containing 0.75% available iodine. These agents generally gave a statistically significant greater reduction in number of bacteria released from hands compared to other agents. The 4% chlorhexidine gluconate product also had a residual effect, significantly reducing the number of bacteria released from hands with successive hand washes. Other agents, with active ingredients that included Irgasan DP 300, *para*-chloro-*meta*-xylenol (PCMX), trichlorocarbanilide

or tribromosalicylanilide, in various formulations, were in general no better than a non-germicidal soap wash. This also applied to low and intermediate strength iodophor products and a 2% chlorhexidine gluconate product. Because the evaluation of the latter agents against the resident microflora depended on the growth of this microflora on Baird-Parker medium, the results cannot be considered conclusive.

The use of hand dipping procedures as a method of hand hygiene was tested with quateruary ammonium compound (QAC, 930 p.p.m.), hypochlorite (50 p.p.m. available chlorine) and iodophor (25 p.p.m. available iodine). Hand dipping was not considered as valuable as the hand washes to achieve reduction in microorganisms released from hands, however the QAC dip did show some improvement over other dip techniques. In contrast, the use of protective barrier creams on hands showed that they might be a means of controlling bacteria release from hands. The use of these agents as an adjunct for hand hygiene requires further study.

The 4% chlorhexidine gluconate liquid detergent (Hibitane ®, Ayerst Laboratories, Montreal) is a product produced for hospital and clinical use. At present, its cost might be a limiting factor to its acceptance for use by food handlers. The iodophor product containing 0.75% available iodine (Prepodyne or "Tamed Iodine Scrub" ®, West Chemicals Ltd., Montreal) is also a product used for medical practice (Prepodyne), but it is also marketed for use by food

handlers ("Tamed Iodine Scrub"). The iodophor product creates some user resistance, recorded in our studies, as well as by other workers (Davis, 1971; Saggers and Stewart, 1964). Further study of the intermediate strength iodophor products as well as the 2% chlorhexidine gluconate product could be valuable for conclusive recommendations regarding hand germicides for food handlers.

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October 12, 1983

Earl O. Wright
Executive Secretary & Managing Editor
Box 701
Ames, Iowa 50010-0701
U.S.A.

Dear Earl:

Arif Sheena and I have four papers that have been published, or they have been accepted for publication in the Journal of Food Protection. These papers are based on the research that Arif Sheena did toward his Ph.D. At this time, he is submitting his thesis for examination, and it consists of the four papers (slightly modified to fit the thesis requirement format), plus a review of the pertinent literature and a fifth research paper that has yet to be submitted for publication.

At the University of Alberta, we have a requirement that all theses be sent to our national library for microfilming, presumably for circulation and/or sale to interested groups. It appears that they will not do the microfilming unless appropriate copyright releases have been obtained. My request is therefore that the IAMFES should release their copyright on these papers, for the purpose outlined above. The papers that are involved are:

Sheena, A.Z. and M.E. Stiles.
"Efficacy of Germicidal Hand Wash Agents in Hygienic Hand Disinfection". J. Food Protection 45:713-720 (June, 1982).

"Immediate and Residual (Substantive) Efficacy of Germicidal Hand Wash Agents". J. Food Protection 46:629-632 (July, 1983).

"Efficacy of Germicidal Hand Wash Agents Against Transient Bacteria Inoculated onto Hands". J. Food Protection 46:722-727 (August, 1983).

"Comparison of Barrier Creams and Germicides for Hand Hygiene". J. Food Protection (In Press).

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I look forward to receiving your reply. If you require further clarification and wish to contact me by telephone, please call me at (403)432-5239.

Best wishes

Mike Stiles

Michael E. Stiles
Professor
Department of Foods and Nutrition

MES/as

Dear Mike —

With proper credit line you may use the papers as you have stated.

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FROM Dr. M.E. Stiles, Professor
Department of Foods and Nutrition
308 Home Economics Building

ATTENTION: Audrey

Re: Copyright regarding Arif Sheena's thesis

Further to our conversation, it appears that the copyright on the papers published by Arif Sheena and myself in the Journal of Food Protection is held by the International Association of Milk, Food and Environmental Sanitarians. As a result, I am writing under separate cover to seek their release from any restrictions for Mr. Sheena's thesis. For my part, as co-author of the papers, I have no objection to their use as part of the thesis.

M. E. Stiles

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